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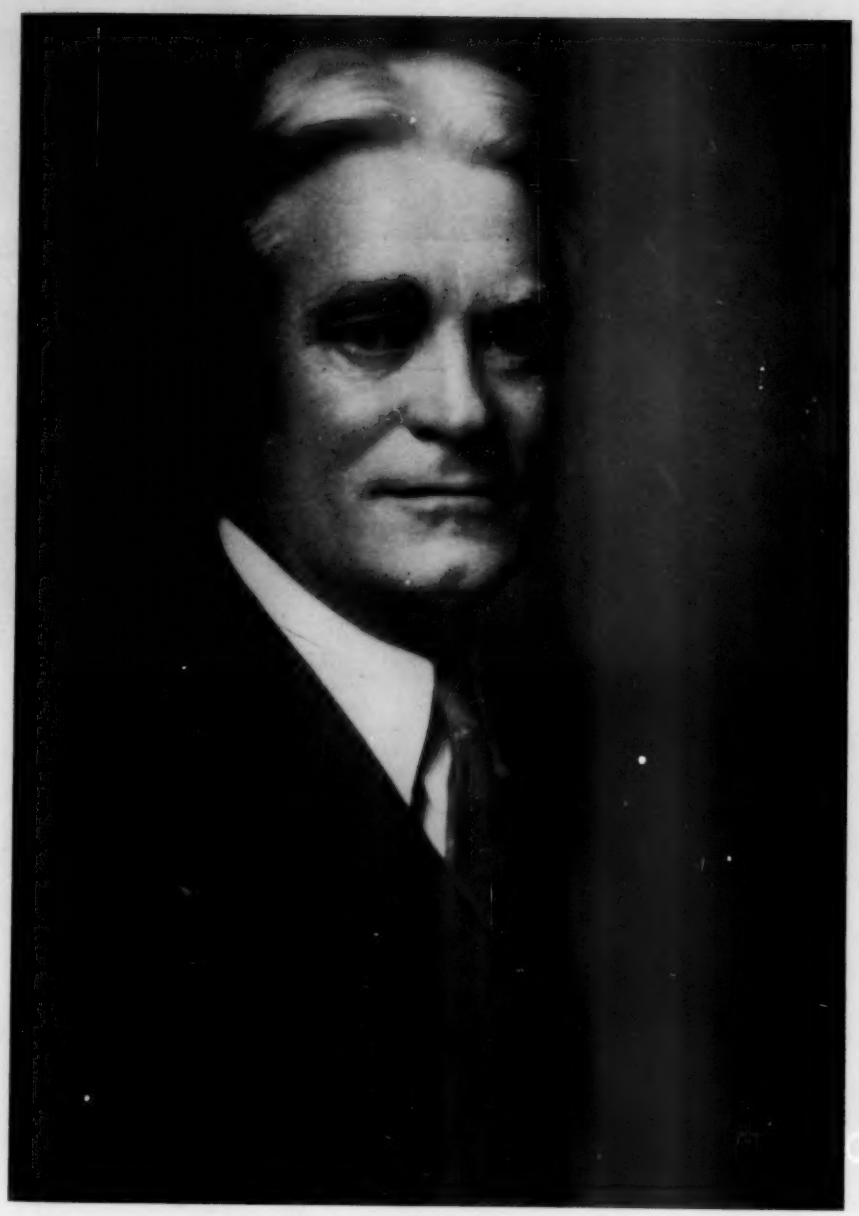

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PLANT PHYSIOLOGY

APRIL, 1945

RESPIRATION AND INTERNAL GAS CONTENT OF INJURED SWEET-POTATO ROOTS

T. M. WHITEMAN AND H. A. SCHOMER

(WITH THREE FIGURES)

The present work concerning the effect of injury on the respiration and gas content of sweet-potato roots was carried on in conjunction with a more detailed study related to the handling of the crop after harvest. More or less injury is likely to occur during the handling of any crop but sweet potatoes are especially susceptible to spoilage if skinned or bruised. Since the effect of injury during the handling of sweet potatoes after harvest involves the physiology of the roots, it seemed desirable to determine whether the increased respiration obtained with injured sweet potatoes is due primarily to the stimulation caused by wounding or merely to the facilitation of gaseous exchange through the open, wounded areas.

Statements concerning the notable increase in the evolution of carbon dioxide following the wounding of plant tissues have usually been general or somewhat ambiguous. MILLER (**6**), in alluding to the work of various experimenters, has reported that this increase "is probably due to the escape of the gas that has accumulated in the intercellular spaces of the tissues"; that "one cause" of the increased respiratory activity of the tissues following wounding "is apparently the facilitation of gaseous exchange"; and that "the increase in respiration rate due to injury is due primarily to an acceleration of the gaseous exchange between the tissues and the surrounding air." This is taken to mean that "such increase is largely due to mechanically facilitating the exchange of gases, rather than to direct wound stimulation," as noted by JOHNSTONE (**4**). HOPKINS (**3**) found an increase in sugars following wounding, and states that "the augmentation of respiration which follows wounding can be explained logically on the basis of the increase in the sugar content of the cells, although it is pointed out that because of other factors the curves for respiration and sugar content will not be exactly parallel." According to MILLER (**6**) "the absorption of oxygen is also rendered more favorable by the freer access of air across the tissues due to injury."

Methods

The two experiments reported here were made on Big Stem Jersey sweet potatoes dug on October 15, 1943, at Millersville, Md. They were transported by truck to the Plant Industry Station, Beltsville, Md., and were stored in a sweet-potato curing house the same day as dug. They were cured for 13 days at 85° F. with a relative humidity of about 85 per cent. Except for a slight difference in the storage treatment previous to the respiration studies, the handling of the roots and the entire experimental plan were identical in the two tests reported here.

The sweet potatoes in test 1, after curing, were stored at 55° F. on October 28 and moved to 70° on November 11. Respiration was determined between November 15 and 20.

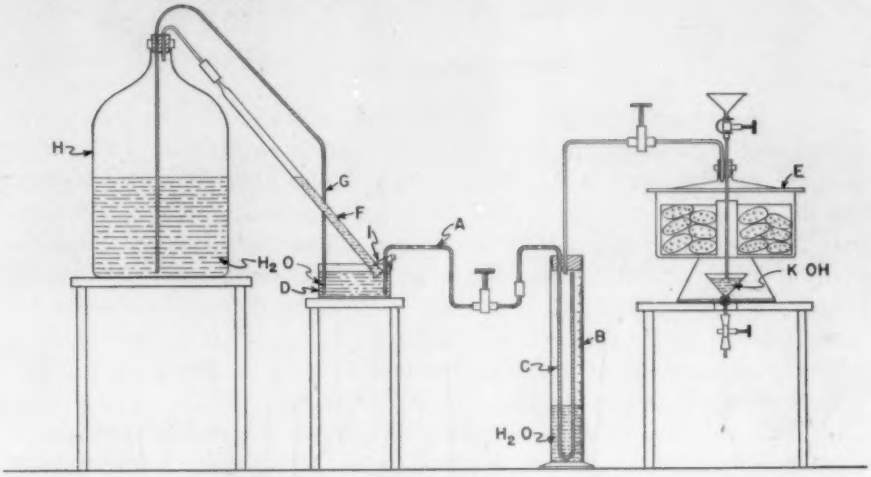

FIG. 1. A static-absorption respirometer.

The sweet potatoes used in test 2 were stored at 60° F. on October 28 and moved to 70° on November 27. Respiration was determined between November 29 and December 4.

The equipment with which respiration was determined was essentially the HALLER-ROSE (**2**) modification of the MAGNESS-DIEHL (**5**) apparatus (fig. 1). By the use of this apparatus both the carbon dioxide evolved and the oxygen taken up by the respiring product are determined; only values for the former are reported here. However, the water supply tube (A) to the oxygen cylinder (B) was extended to form a U-tube (C) so that even small quantities of oxygen may be replaced, as soon as used, by water. The oxygen cylinder was adjusted so that whenever this was replaced, the water level in the U-tube always stood exactly at the top of the open end. This modification takes care of negative or positive pressures in the desiccator (E) without any break occurring in the siphon. To prevent any great fluctuation in the water level of the secondary reservoir (D) a vessel of about 8 inches in diameter was substituted for the beaker used in the HALLER-ROSE

apparatus. A third modification consisted in using a larger siphon cut-off tube (F) of about 10 mm. diameter. If this is set at an angle, the water flows out of it very quickly after the water level falls below it. This occurs against the increasing negative pressure in the primary reservoir (H). The letter (I) indicates the adjusting string for the cut-off tube; (G) is the primary siphon tube; and (A), the secondary siphon tube. The operation of this apparatus has been described by HALLER and ROSE (2).

The apparatus used for the extraction of gases from the sweet potato roots will be described in a later publication by the junior author. Briefly, its principle involves vacuum extraction of the total amount of atmosphere and/or gas in solution from the entire sample which is placed in a CO_2-free liquid. By some of the older methods of gas extraction the volume of air extracted has not usually been the total volume but only a portion of it. From this extracted portion an aliquot has been analyzed. By previous trials in the use of the method employed in these tests it was found that the period of extraction and the degree of vacuum affected not only the total amount of gas extracted but also its composition.

The wounding consisted in cutting off the proximal end of each root, making cuts averaging 37.4 mm. in diameter in the 8 lots in test 1 and 36.7 mm. on those in test 2. Through the cut ends a cork borer of 5 mm. diameter was inserted three times to a uniform depth of 55 mm. The removed cut ends and the cork-borer plugs were discarded.

In test 1, the mean cut area of the 8 wounded lots was $10.95 \pm .419$ sq.cm. The cut area for the four not-sealed lots averaged $11.48 \pm .63$ sq.cm. and for the sealed lots $10.41 \pm .54$ sq.cm. In test 2, the mean area for all wounded lots was $10.45 \pm .27$ sq.cm. The cut area for the not-sealed lots was $10.55 \pm .28$ sq.cm. and for the sealed lots, $10.36 \pm .45$ sq.cm. The internal wounding with the cork borer amounted to 25.9 sq.cm. for each root, or 103.6 sq.cm. for each lot of 4 roots. Since the latter figure was uniform throughout the two tests, the only variation in the areas wounded was that of the cut end. The mean wounded areas of the 4 sealed and the 4 not-sealed lots do not differ statistically in either test. Neither do they differ statistically for any comparison of individual not-sealed versus sealed lots in either test.

All respiration determinations were made at 70° F. Sound, unblemished sweet potatoes were selected and divided into 10 lots of 4 roots each. The lots for the various treatments were picked at random. Respiration was run for 2 days on these 10 not-wounded lots. On the third day respiration measurements were suspended on 8 of these lots sufficiently long to wound them. In each test there were 4 lots of roots in which the wounded areas were left exposed to the desiccator atmosphere and 4 lots in which the cut ends were completely sealed over with ordinary carpenter's caulking compound. Respiratory activity was recorded for 3 successive days following wounding, the results for both the sealed and the not-sealed lots being averages of 4 lots one day after wounding, of 3 lots 2 days after wounding, and

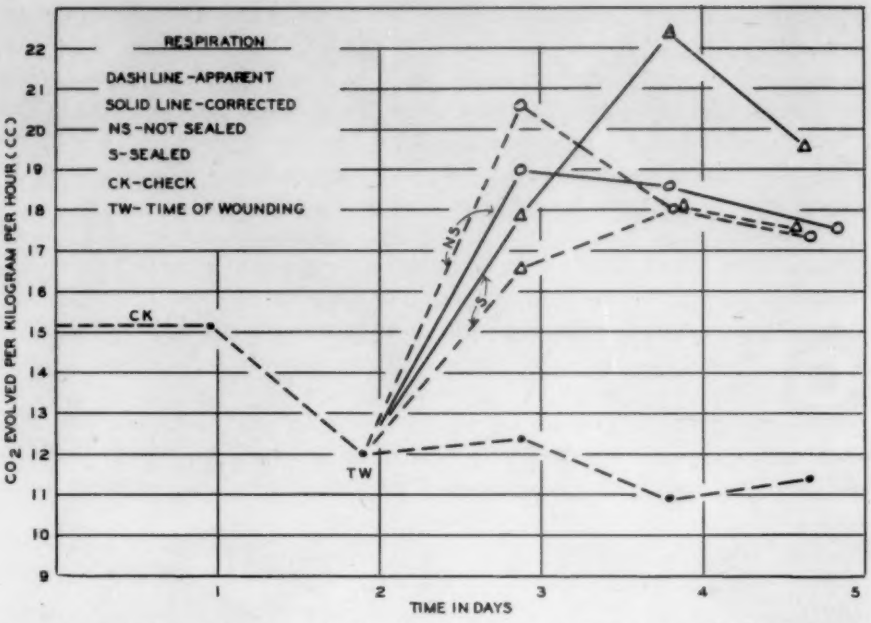

FIG. 2. Apparent and corrected respiration of wounded (sealed and not-sealed) and not-wounded Big Stem Jersey sweet potatoes, test 1.

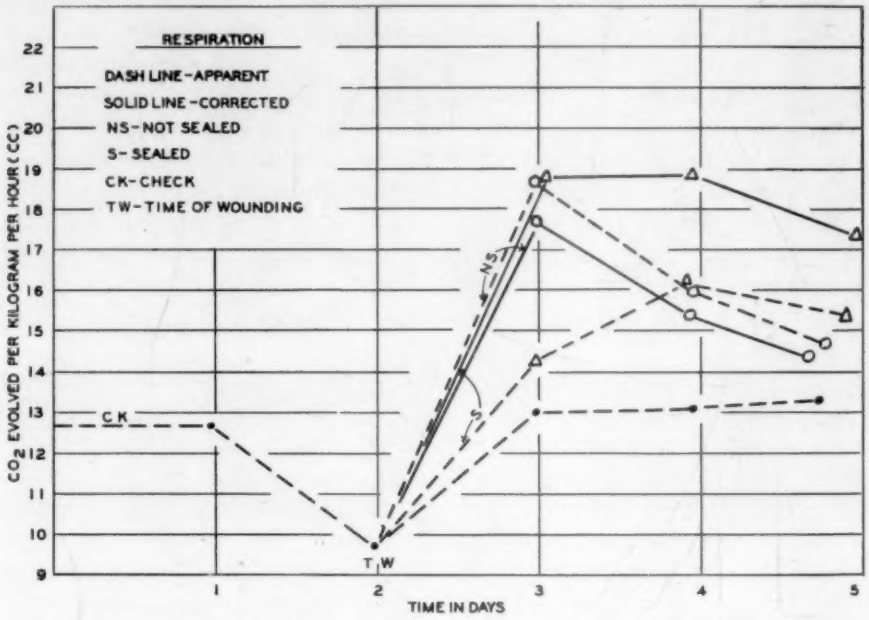

FIG. 3. Apparent and corrected respiration of wounded (sealed and not-sealed) and not-wounded Big Stem Jersey sweet potatoes, test 2.

of 2 lots 3 days after wounding since one lot of each group was stopped each day for internal gas determinations. The weights of individual lots in test 1 ranged from 661 to 983 gm. and in test 2, from 577 to 848 gm., the averages for those in the two tests being 774 and 735 gm., respectively.

In the following discussion, respiration as measured by the direct absorption of CO_2 in the respiration apparatus is referred to as apparent respiration. That which has been corrected to include the CO_2 of the internal gases is referred to as true (corrected) respiration.

Analyses of internal gas were made on not-wounded roots at the start of the respiration studies and on each of the three successive days of the experiment. The analyses on the first and third days were on lots from the respiration chambers on which respiratory data had been taken. The other analyses were on roots from baskets in the room in which the experiment was being performed.

The wounded roots on which respiration had been determined were analyzed for internal gases as follows: One not-sealed and one sealed lot were analyzed both 1 and 2 days after wounding, and two not-sealed and two sealed lots 3 days after wounding. The extracted CO_2 in cubic centimeters minus that for the check was calculated as kg. per hr. values for these lots and such calculated rates were added to the apparent respiration results, as shown in figures 2 and 3.

Results

Internal gases

Table I shows the total volume of internal gases extracted in 1 hour from the various lots of sweet-potato roots and the corresponding amounts of CO_2 and O_2 expressed in cubic centimeters per kilogram of tissue. It is evident from the table that unusually large amounts of gas were obtained by the method of extraction used. It was observed with each lot of roots that practically no additional gas was obtained from them after a 45-minute extraction, so a 60-minute period of extraction was arbitrarily adopted for all samples.

Corrections for variation in gas volumes due to different temperatures and pressures were found to be negligible and, consequently, are not reported.

From table II it can be seen that the analyses of internal gases from the 8 not-wounded check lots reported showed the average of these to be intermediate between the averages of the 6 wounded sealed lots and those of the 6 wounded not-sealed lots. The wounded sealed lots averaged almost twice as high a percentage of CO_2 as the average of the checks, and that with this increase there was also a concomitant decrease in percentage of O_2. That the average percentage of CO_2 was lower in the wounded not-sealed lots than in the check is attributed to a loss of some of the original CO_2.

In test 1 the not-wounded roots on the day of wounding contained 51.3 cc. of CO_2 per kg. of tissue (table I). This figure was used in all calculations

TABLE I

VOLUME AND COMPOSITION (CO_2 AND O_2) OF INTERNAL GAS EXTRACTED IN 1 HOUR PER KILOGRAM OF BIG STEM JERSEY SWEET-POTATO ROOT TISSUE*

DAYS AFTER WOUNDING	RESULTS EXPRESSED IN	NOT-WOUNDED ROOTS								
		TEST 1			TEST 2			TEST 2— OPEN BASKET LOTS; RESPIRATION NOT MEASURED		
		GAS VOLUME	CO_2	O_2	GAS VOLUME	CO_2	O_2	GAS VOLUME	CO_2	O_2
0	cc.	210.2	51.3	28.6	208.0	59.5	24.5	189.7	50.1	24.8
	%	.....	24.4	13.6	.....	28.6	11.8	.....	26.4	13.1
1	cc.	.....	.....	.....	.....	.....	.....	197.6	52.9	25.7
	%	.....	.....	.....	.....	.....	.....	.....	26.8	13.0
2	cc.	.....	.....	.....	.....	.....	.....	192.4	53.9	23.9
	%	.....	.....	.....	.....	.....	.....	.....	28.0	12.4
3	cc.	235.4	87.9	23.5	202.3	61.7	24.1	205.2	68.5	23.0
	%	.....	37.3	10.0	.....	30.5	11.9	.....	33.4	11.2

* Each figure in this table represents gas extracted from lots of 4 roots each, on which, except where noted, respiration was previously determined as shown in figures 2 and 3.

TABLE I—(*Continued*)

VOLUME AND COMPOSITION (CO_2 AND O_2) OF INTERNAL GAS EXTRACTED IN 1 HOUR PER KILOGRAM OF BIG STEM JERSEY SWEET-POTATO ROOT TISSUE

DAYS AFTER WOUNDING	RESULTS EXPRESSED IN	WOUNDED ROOTS											
		TEST 1						TEST 2					
		NOT-SEALED			SEALED			NOT-SEALED			SEALED		
		GAS VOLUME	CO_2	O_2	GAS VOLUME	CO_2	O_2	GAS VOLUME	CO_2	O_2	GAS VOLUME	CO_2	O_2
0	cc.	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
	%	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
1	cc.	189.9	15.6	34.2	249.7	79.4	30.4	181.5	31.6	26.5	300.0	160.2	19.8
	%	.....	8.2	18.0	.....	31.8	12.2	.....	17.4	14.6	.....	53.4	6.6
2	cc.	249.6	79.9	31.9	460.4	254.6	42.3	175.1	24.5	28.7	318.8	176.9	21.4
	%	.....	32.0	12.8	.....	55.3	9.2	.....	14.0	16.4	.....	55.5	6.7
3	cc.	224.3	62.8	30.2	338.5	180.0	19.1	178.6	30.9	24.3	355.5	199.2	16.5
	%	.....	28.0	13.5	.....	53.2	5.6	.....	17.3	13.6	.....	56.0	4.6

of true respiration values in this test. True respiration has been defined above as the apparent respiration plus the CO_2 of the internal gases. In the not-sealed wounded lots of this test, the CO_2 per kilogram of tissue decreased below the check but increased, as compared with the check, on the second and third days after the wounding date. The difference in CO_2 between the 51.3 cc./kg. of the check and the 15.6, 79.9, and 62.8 cc./kg., divided by the number of hours that these lots had respired beyond the time of wounding, gives the correction in cc./kg.hr. that must be added to or subtracted from the figure for apparent respiration. It will be noted that in the sealed lots of test 1 the cc./kg. values for CO_2 increased considerably one, two, and three days after wounding.

In test 2 the CO_2 per kilogram extracted from the check lot on the day of wounding was 59.5 cc. (table I). The amount of CO_2 obtained from the not-sealed lots one, two, and three days after wounding was in all cases less

TABLE II

AVERAGE PERCENTAGE OF CO_2 AND O_2 IN GAS EXTRACTED FROM 8 NOT-WOUNDED LOTS AND 6 LOTS EACH OF WOUNDED SEALED AND WOUNDED NOT-SEALED BIG STEM JERSEY SWEET POTATOES

TREATMENT	CO_2	O_2
	%	%
Not wounded	29.6	12.1
Sealed (wounded)	51.9	7.4
Not-sealed (wounded)	20.5	14.7

than the check; that obtained from the sealed lots was approximately three times as much as the check.

Since the amount of CO_2 per kilogram of the final check lot in test 1 showed an increase of 36.6 cc. (87.9–51.3) over that analyzed on the day of wounding, it was decided that in test 2 a check sample should be analyzed each day. Accordingly, the "open basket," not-wounded lots reported in table I were analyzed and found to contain 50.1 cc. of CO_2 per kilogram on the date of wounding, with slight increases above this amount 1, 2, and 3 days after the date of wounding.

The average O_2 content of the four not-wounded lots held in an open basket and the four check lots in tests 1 and 2 (table I), was 24.8 cc. per kilogram of tissue; for the not-sealed lots in test 1, 32.1 cc.; and for comparable lots in test 2, 26.5 cc.

APPARENT RESPIRATION

The respiration curves for the first two days of each test (check in figures 2 and 3) show average values for each ten lots. During the first two days the respiratory rates of the 10 check lots in both tests 1 and 2 (figs. 2, 3) decreased; for the remaining 3-day period these rates remained fairly constant in test 1 and rose slightly in test 2.

In test 1 (fig. 2) the average respiratory rate for the four not-sealed

wounded lots (NS) was 4.0 cc. per kg. per hr. higher than the average rate for the sealed lots (S), during the day following their wounding. During the second day both the not-sealed and sealed gave off equal amounts of CO_2 per kilogram per hour; and on the third day after wounding there was a difference of only 0.2 cc. per kg. per hr. between the not-sealed and the sealed lots.

In test 2 the relationship between the not-sealed lots and the sealed lots was the same as that in test 1, the only difference being that in test 2 all of the values were slightly lower than in test 1.

True respiration

The method of obtaining the true respiration results has been explained by the use of figures in the section headed "Internal gases."

In test 1 the corrections above or below the not-wounded check of 51.3 cc. CO_2 per kilogram obtained by gas extraction amounted to + 1.22 in the sealed lot (S) and – 1.55 cc. per kg. per hr. in the not-sealed lot (NS) one day after wounding, + 4.35 and + 0.61 for these respective lots 2 days after wounding, and + 1.99 and + 0.26 cc. CO_2 per kg. per hr. for the sealed and the not-sealed lots, respectively, 3 days after wounding.

In test 2 the not-wounded check lot (Ck) on the date of wounding gave a CO_2 content of 59.5 cc. per kg. The gas analysis of the wounded lots in terms of cc. CO_2 per kg. per hr. was calculated to be + 4.43 and – 1.04 for the sealed (S) and the not-sealed (NS) lots, respectively, one day after being wounded; + 2.70 and – 0.68 cc. the second day; and + 2.03 and – 0.37 cc. the third day after wounding.

Discussion

Hopkins (**3**) found that wounding of potatoes stimulated sugar formation, and the increase in sugar was accompanied by a rise in the respiratory rate. This increase in sugar was thought to be brought about by activities leading to callus formation.

Miller (**6**) states that "one cause" of the increased respiratory activity of tissues following their injury "is apparently the facilitation of gaseous exchange," but the results of this study indicate that the real cause of the increased activity is wound stimulation. By the use of the customary methods of determining respiration, the entire increased quantity of CO_2 evolved in the not-sealed lots, due to wounding, can be measured because of the freer interchange of gases through these exposed surfaces. As previously mentioned, however, this measured increase may also include some of the CO_2 present before wounding. In sweet-potato roots with sealed, wounded surfaces the customary methods of determining respiratory rates do not measure the entire increase in CO_2 from respiration, due to wound stimulation, because of the comparative slowness of diffusion through the normal skin.

Gas absorption in cell sap

Whereas the weight of CO_2 that can be dissolved in 100 gm. of water at 70° F. is about .164 gm. per 100 cc., when the sum of the partial pressure of

the gas plus the aqueous tension is 760 mm., the amount under the conditions of this experiment would be considerably less because of the low partial pressure of CO_2 in the desiccator atmosphere. However, unless a fairly large amount of CO_2 goes into solution in the sweet-potato roots it would be difficult to explain both the presence of such a large volume of gas and its high CO_2 content in the sealed lots subsequent to wounding. It is possible that instead of forming carbonic acid the CO_2 combines with other cell sap constituents. If this is true the cell sap in the sealed lots may very well retain larger quantities of CO_2 at a temperature of 70° F. than if the CO_2 were present in solution only as carbonic acid.

Since the true respiratory rates of the sealed lots increased for 1 and 2 days after wounding it is doubtful that the accumulation of CO_2 within the roots could have had any retarding effect on respiration. If, on the other hand, the CO_2 of the internal atmosphere has retarded respiration here, then this effect is evidently masked by the unusually large amount given off by the wounded roots.

In contrast to the sealed lots, those not sealed would hardly be expected to retain CO_2 to the extent of the former. Where the gas can escape only through normal, not-wounded tissue, as with the sealed lots, the buildup of CO_2 as a result of increased respiration due to wounding would be rapid and its release slow. That the CO_2 emanates slowly through the skin is shown in tests 1 and 2, both by the lower apparent and true respiratory rates of the sealed lots one day after wounding and by the maintenance of a considerably higher true respiratory rate 3 days after wounding.

Wound stimulation versus freer interchange of gases

In the not-sealed lots in tests 1 and 2 (figs. 2, 3) the apparent respiratory rates were considerably higher one day after wounding than in the sealed lots. According to generally accepted methods of measuring respiration, it would naturally appear that this increase of the not-sealed over the sealed lots was due merely to the CO_2 escaping easily and to the ease of its replacement by O_2. The conclusion to be drawn from a comparison of these lots, by old standards, would then be that about 50 per cent. of the increase of respiration 1 day after wounding in test 1 was due to wounding (S) and about 50 per cent. to freer interchange of gases (NS). In test 2 this increase over the check would be roughly in the proportion of 40 per cent. due to wounding (S) and 60 per cent. because of the ease of escape of the gases through the cut ends (NS). When, however, the CO_2 extracted from the roots is added to the apparent respiratory rate it becomes clear that the true respiratory rates of the sealed (S) and the not-sealed (NS) lots (in both tests) are essentially the same. Therefore all or practically all of the increase in respiratory activity following wounding is due directly to this injuring of the cellular structure.

Respiration in the sealed lots during the day following wounding had been going on at the same rate as in the not sealed lots, but merely had not

been measured with the respiration apparatus used. As is evidenced by the lower O_2 content of the sealed lots, the interchange of gases through the skin of these lots had no doubt been slower than if the cut surfaces had been exposed, even though the respiratory rate increased the day after wounding. During the retardation period the CO_2 was evolved at a much faster rate due to wounding; and because its escape was inhibited physically it probably was "forced" into solution. Contrasted with this situation is the easy and rapid escape of CO_2 in the not-sealed lots, not only the additional CO_2 formed by the stimulation of wounding (see NS lines, figures 2 and 3) but also part of that already in the roots previous to wounding, which is obvious when comparison is made of the not-sealed lots in table I with their respective test checks and also with the open-basket checks. By comparing lines NS, apparent and corrected, in figures 2 and 3, it is seen that the corrected or true respiratory rate is lower than the apparent rate. This is due to the escape and measurement in respiration of a small part of the original CO_2 content present before wounding.

The results presented here tend to substantiate to some extent MILLER'S (**6**) assertion that "the absorption of oxygen is also rendered more favorable by the freer access of air across the tissues due to injury" since the not-sealed lots in test 1 contained on an average 7.3 cc. of O_2 per kilogram of tissue, and those in test 2, 1.7 cc. per kilogram, more than the average for all check lots.

WOUND STIMULATION AND SUBERIZATION

Thus far the discussion has dealt primarily with the results during the day following wounding. In both tests the results on the second and third days were sufficiently similar that they can be considered simultaneously.

In the not-sealed lots the stimulatory effect of wounding decreased between 1 and 2 days after wounding to the extent that both the apparent and the true respiratory rates declined. With the wounds exposed to an atmosphere of 21 per cent. O_2 in the desiccator, it is assumed that suberization probably started during the second day after wounding, resulting in (1), a slower evolution of CO_2 and (2), a retarding of the former extremely free interchange of gases. Under favorable conditions of temperature and humidity, such as prevailed in these experiments, ARTSCHWAGER (**1**) reported positive tests for suberin in sweet-potato tissue 24 hours after wounding.

In the sealed lots, as shown by figures 2 and 3, both the apparent and true respiratory rates continued to increase during the second day after wounding and declined on the third day. With the continued evolution and accumulation of CO_2 and its slow escape into the atmosphere there is noted a wide spread between the apparent and true respiratory rates. The results from the sealed lots might be interpreted to mean that the suberization processes were retarded for 2 days after wounding due to sealing.

Conclusions

Insofar as the writers are aware the respiration studies that have been

reported for various plant parts have dealt only with apparent respiration. From the results of the tests now being reported it is concluded:

1. The addition of the extracted internal CO_2 to the *apparent* respiration values gave the *true* respiration of the product.

2. The increase in the *apparent* respiratory rates of both sealed and not-sealed wounded sweet-potato roots which immediately followed wounding was due entirely to wound stimulation, the superficial differences between these treatments having resulted from incomplete measurements. When the CO_2 of the internal gases was added to that of apparent respiration, the *true* respiration value was obtained and was the same for both the sealed and not-sealed lots.

3. The increase in internal CO_2 content in the sealed lots of sweet potatoes was due entirely to wound stimulation.

4. The apparent respiration of wounded, not-sealed roots was somewhat higher than true respiration, due to the escape of some of the CO_2 that was present before wounding.

5. In the sealed lots a large amount of the CO_2 that is rapidly formed after wounding sweet-potato tissue went into solution as carbonic acid or combined in some other form with cell sap constituents, since the wounded area was sealed over so as to prevent its escape through this channel.

6. To obtain a true measurement of the respiratory rate of any plant organ or tissue the apparent rate should be corrected to include any change between the initial and the final internal content of CO_2.

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GROWTH AND NUTRIENT RESPONSES OF LITTLE TURKISH TOBACCO TO LONG AND SHORT PHOTOPERIODS

RAYMOND A. DENNISON

(WITH THREE FIGURES)

Introduction

Physiological studies of strongly photoperiodic species in long and short day have hitherto dealt predominantly with the effect of day length upon development rather than upon growth. In photoperiodic species which flower in only one of two contrasted day lengths, it becomes difficult to compare or interpret data on growth due to simultaneous developmental differences of plants grown in unlike photoperiods. The use of so-called day-neutral species whose flowering response is relatively independent of day length facilitates recognition of growth as opposed to developmental responses caused by the photoperiod. Though data are available on the photoperiodic responses of certain day-neutral forms such as the tomato, it nevertheless seemed worthwhile to undertake a study of growth responses of a species having a distinctly different structural habit from those types studied heretofore.

Preliminary tests with Little Turkish Tobacco, a dwarf form of *Nicotiana tabacum,* revealed that vegetative growth and flowering were both fairly uniform over a wide range of day length. It was found possible to maintain approximate developmental similarity and date of flowering of Little Turkish Tobacco in day lengths as short as six and one-half hours on the one hand and as long as fourteen hours on the other. The ontogenic cycle thus appeared to be comparable over wide photoperiodic ranges even though differences in photoperiod produced certain contrasts in general growth habit. In order to determine the structural and metabolic effects of differences in day length, the developmental cycle of Little Turkish Tobacco was traced from the early vegetative to the fruiting stage in long and short days respectively.

Methods

Seeds of *Nicotiana tabacum,* variety Little Turkish, were planted in germination trays on August 6, 1941. The seedlings were transplanted on September 14 into two-gallon glazed pots, each of which contained an equal weight of inert silica gravel ($\frac{1}{8}$- to $\frac{1}{4}$-inch size) which had been washed free of solutes. Single seedlings were transplanted to 40 pots when they were approximately one inch tall. The plants were divided into two groups of 20 pots each. The short-day group received normal daylight only between 9:30 A.M. and 4:00 P.M., namely a daily light period of $6\frac{1}{2}$ hours. The remainder of the time they were covered with completely light-proof black sateen. The long-day group received the full length of normal daylight plus additional electric illumination in the evenings in order to extend the

daily light period to 14 hours. For the artificial lighting, incandescent lamps provided a light intensity of approximately 200 foot-candles at the top of the plants, as determined by a photoelectric light meter.

The plants were grown on WITHROW's (moderate light) nutrient solution (**27**) which contained traces of the minor elements iron, boron, zinc and manganese. Culture pots were completely drained on alternate days and a fresh nutrient solution added to maintain uniformity of supply. The exact volume of solution drained from each pot was recorded in order to determine the water consumption for each two-day period. Solution samples drained from each group of plants were preserved in Pyrex flasks and later used for determination of residual salts. Continuous records were kept of daily light conditions, temperature, and the relative humidity.

The plants were sampled for chemical analyses at four different phases of growth. The first samples were taken 24 days after the plants had been transplanted to the pots at which time the stems had commenced to elongate. The second sampling was made 44 days after transplanting at the time the plants exhibited their greatest vegetative growth. The third set of samples was taken 59 days after transplanting, about 4 days after the floral buds were first visible at the stem apex in both long- and short-day groups. The fourth sampling was taken 79 days after transplanting when inflorescences had fully developed and a number of young fruit capsules had already formed in both groups.

Each plant was separated into seven portions for analyses. The stem was divided into three approximately equal segments designated as upper, middle, and lower stem according to their original location on the intact plant. The leaves were separated from each of these stem segments and analyzed independently. For the fourth sampling, the inflorescence was severed from the rest of the plant and analyzed separately. The roots were carefully freed of gravel, rinsed in distilled water, and dried between sheets of absorbent paper. The root system was analyzed as a unit. The contour of the fresh leaves was carefully drawn and the leaf area determined later by measuring the outline with a planimeter. Fresh weights were recorded for each portion of the plant and tissues were then comminuted. Samples for sugar determinations were killed in boiling 80 per cent. alcohol. Samples for nitrogen and ash determinations were rapidly dried at 100° C. in an electric oven. Dry weights were determined by drying to constant weight. Ash weights were obtained by slow ignition of dry samples in the open flame of a Bunsen burner and weighing to constant weight.

The material preserved in alcohol was extracted several times with 80 per cent. alcohol (**23**). Aliquots of the clarified extract were hydrolyzed with concentrated hydrochloric acid for the determination of soluble carbohydrates by the FOLIN-MALMROS method (**3**) and the results expressed as glucose. The residue from the alcoholic extraction was hydrolyzed by refluxing for three hours with dilute hydrochloric acid and further analyzed as polysaccharides (**2**). Total nitrogen, including nitrates, was determined

from the dried tissue. Digestion was carried out according to the salicylic acid sodium thiosulphate method (**9**), using metallic selenium as the catalyst (**1**). Distillation was according to PREGL's method (**16**). Soluble nitrogen was extracted from the dried material and the total soluble nitrogen was determined by digesting with reduced iron powder and sulphuric acid (**9**) and the distillation was performed the same as for total nitrogen. Ammonia from the distillation was received in N/14 HCl and the excess acid was then titrated with N/14 NaOH using methyl red as an indicator.

Data and discussion

Both long- and short-day groups were healthy and all plants in each respective set were uniform in appearance as well as in rate of development. No leaves abscissed in either the long- or short-day group. The basal leaves

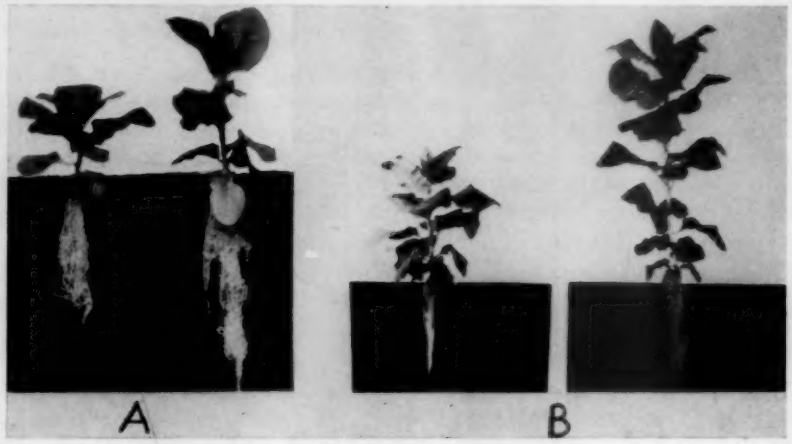

FIG. 1. Representative plants grown in 6½- and 14-hour day lengths. A, Rosette stage; B, Elongation stage. Plant grown in short day shown at left in A and B.

persisted in a healthy, functional condition in both groups until the end of the experiment. There was no evidence of virus, insect, or fungus infection at any time. A conspicuous difference in the appearance of the short-day from the long-day group of plants was observed during the vegetative growth. The leaves of the short-day group were lighter green in color until the fifty-second day, three days previous to the appearance of flower buds. Three days after the flower buds became visible (age 58 days), there was no noticeable difference in the intensity of color in the leaves of the two groups. MURNEEK (**11, 13**), VIRTANEN (**25**), and others (**24**) have observed a similar change in color which is attributable to increased carotin and xanthophyll content occurring during the transition from the vegetative to the reproductive state.

The photographs (figs. 1, 2) of the two series of plants disclose a slower growth rate in short-day plants. Stem elongation of the short-day plants was slower throughout the growth cycle but the number of nodes was greater

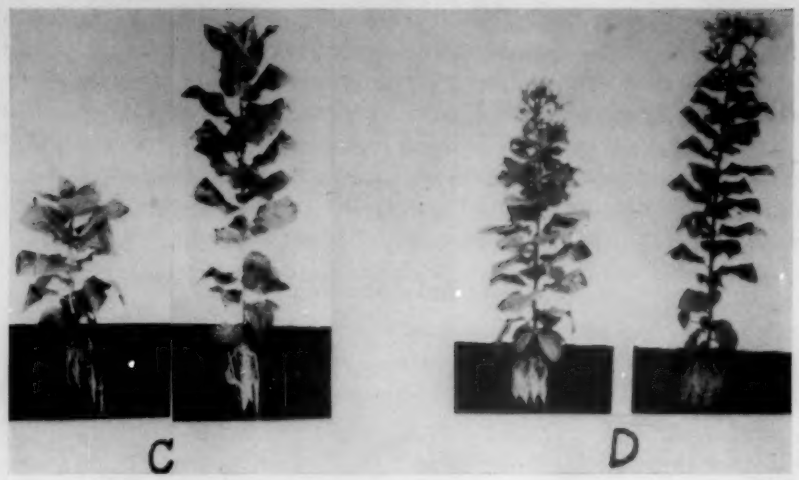

FIG. 2. Representative plants grown in 6½- and 14-hour day lengths. C, Flower bud stage; D, Blooming and early fruiting stage. Plant grown in short day shown at left in C and D.

than in long-day plants. At the beginning of capsule formation, the average height in centimeters of the short-day plants was 94 compared to 138 for the long-day group. Short-day plants produced a total of 47 leaves compared to 40 leaves for the long-day group. The results appear to confirm the conclusion that the length of the photoperiod controls the rate of increase in the

TABLE I

LEAF AREAS IN SQUARE CENTIMETERS AT THE FOUR PERIODS OF SAMPLING. AGE IN DAYS AFTER TRANSPLANTING

	DAY LENGTH	LEAF AREAS	24 DAYS	44 DAYS	59 DAYS	79 DAYS
			sq.cm.	*sq.cm.*	*sq.cm.*	*sq.cm.*
Upper leaves	Long	Total	129	491	1057	2098
		Average	43	98	132	138
	Short	Total	122	221	773	2125
		Average	41	59	107	105
Middle leaves	Long	Total	171	1178	3535	3800
		Average	53	188	283	310
	Short	Total	136	861	2558	3815
		Average	44	150	256	332
Lower leaves	Long	Total	88	543	1059	2651
		Average	25	68	111	212
	Short	Total	83	504	1511	3744
		Average	24	61	141	238
Entire plant	Long	Total	388	2212	5651	8549
		Average	40	115	188	214
	Short	Total	341	1586	4842	9684
		Average	36	86	173	204

leaf surface during the various periods of growth (**12, 13**). The long-day group had a greater total leaf area than the short-day group until the final sampling, at which time the leaf area in the short-day group exceeded that in the long-day group by 13 per cent. In general, the rate of growth of the plants in terms of dry weight gain increased in proportion to the total leaf area (table I).

The effect of the day length was also reflected in differences between the root systems of the two groups (figs. 1, 2). Root systems of the short-day group were smaller throughout the growth period, as shown by the top/root ratios calculated both on the fresh and dry weight basis. Short-day plants show a larger ratio except at the last sampling (table II). The large top/root ratio for the short-day group, viewed in terms of carbohydrate content at the second sampling, suggests that the major portion of the photosynthate had been utilized by the top and only a small and apparently inadequate fraction had reached the root. The smaller top/root ratios for the

TABLE II

TOP-ROOT RATIO OF PLANTS GROWN IN 14- AND 6½-HOUR DAY LENGTHS. BASED ON FRESH AND DRY WEIGHT IN GRAMS

	Day length	Age in days			
		24	44	59	79
Fresh weight	Long	1.30	4.79	6.55	3.93
	Short	1.75	7.95	8.80	4.07
Dry weight	Long	2.32	6.70	5.15	3.52
	Short	2.90	10.22	5.88	3.48

third sampling indicated not only a better balance in distribution of assimilated materials between the top and root of the short-day group, but also that these plants were approaching the long-day group in composition. At the time of the last sampling, the top/root ratios of the two groups were almost identical.

Flower buds were visible in long- and short-day plants at 55 days and both sets later blossomed simultaneously. Short day length, however, exerts a quantitative effect upon reproduction through differentiation of smaller inflorescences with fewer flowers. At the time of the final sampling, an average of 125 flowers had formed on the short-day plants compared to 185 flowers formed on the long-day plants. Flowers and fruit capsules developed in short day length were noticeably smaller. Individual fruit capsules developed at the termination of the experiment had an average dry weight of 0.08 gram in the short day length compared to 0.14 gram in the long day. Differences in capsule weight were due to a greater number rather than to larger size of seeds in long-day fruits.

Both tops and roots of the long-day group had a greater fresh weight for the first three periods. By the time of the last sampling, however, the fresh weight of the total leaves of the short-day group was 21 per cent.

TABLE III

CONTENT OF LEAF LAMINAE IN MILLIGRAMS PER SQUARE CENTIMETER OF WATER, FRESH WEIGHT, ASH WEIGHT, DRY WEIGHT, TOTAL HYDROLYZABLE CARBOHYDRATES, AND TOTAL NITROGEN

	Days	Water		Ash		Fresh weight		Hydrolyzable carbohydrates		Dry weight		Total nitrogen	
		Long day	Short day	Long day	Short day	Long day	Short day	Long day	Short day	Long day	Short day	Long day	Short day
		mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
Upper leaves	24	19.3	21.8	0.34	0.39	21.4	23.8	0.35	0.21	2.05	1.95	0.12	0.11
	44	22.0	22.9	0.38	0.35	24.4	25.0	0.27	0.17	2.36	2.13	0.15	0.13
	59	20.1	19.9	0.37	0.30	22.7	21.9	0.18	0.16	2.51	1.94	0.17	0.13
	79	21.7	22.2	0.53	0.43	24.9	24.7	0.23	0.39	2.13	2.54	0.16	0.18
Middle leaves	24	21.8	23.7	0.43	0.42	24.0	25.7	0.35	0.20	2.14	2.01	0.09	0.10
	44	23.3	25.8	0.41	0.36	25.4	27.6	0.19	0.09	2.08	1.76	0.12	0.13
	59	23.3	24.4	0.48	0.38	25.8	26.1	0.16	0.13	2.48	1.73	0.14	0.12
	79	22.4	23.0	0.48	0.47	25.1	25.3	0.25	0.36	2.77	2.36	0.14	0.15
Lower leaves	24	23.2	22.8	0.44	0.44	25.1	24.6	0.40	0.19	1.90	1.77	0.03	0.04
	44	25.9	28.6	0.57	0.44	28.3	30.6	0.20	0.08	2.44	1.97	0.08	0.11
	59	26.1	29.2	0.51	0.43	28.2	31.0	0.22	0.11	2.12	1.78	0.07	0.11
	79	24.8	29.0	0.53	0.55	27.1	31.2	0.32	0.47	2.27	2.21	0.17	0.11
Total	24	21.3	22.8	0.40	0.41	23.4	24.7	0.36	0.20	2.06	1.93	0.09	0.09
	44	23.6	26.3	0.44	0.38	25.8	28.2	0.21	0.10	2.23	1.88	0.12	0.12
	59	23.2	25.2	0.47	0.38	25.6	27.0	0.17	0.13	2.42	1.78	0.13	0.12
	79	23.0	25.2	0.51	0.49	25.7	27.5	0.26	0.40	2.70	2.34	0.15	0.14

TABLE IV

FRESH WEIGHT, DRY WEIGHT, AND ASH ANALYSES OF PLANTS GROWN IN 14- AND 6½-HOUR DAY LENGTHS

SEGMENT	DAY LENGTH	FRESH WEIGHT				PERCENTAGE DRY WEIGHT				ASH PERCENTAGE DRY WEIGHT			
		24 DAYS	44 DAYS	59 DAYS	79 DAYS	24 DAYS	44 DAYS	59 DAYS	79 DAYS	24 DAYS	44 DAYS	59 DAYS	79 DAYS
		gm.	*gm.*	*gm.*	*gm.*	%	%	%	%	%	%	%	%
Upper leaves	Long	2.76	11.96	23.96	52.16	9.55	9.67	11.08	12.58	16.49	15.95	15.06	16.90
	Short	2.90	5.53	16.90	52.47	8.20	8.51	8.86	10.30	20.24	16.48	15.63	17.05
Middle leaves	Long	4.10	29.87	91.07	95.52	8.94	8.21	9.64	11.00	19.90	19.60	19.42	17.47
	Short	3.49	23.77	66.80	97.07	7.83	6.40	6.63	9.26	20.79	20.19	21.79	20.04
Lower leaves	Long	2.21	15.37	29.87	71.84	7.55	8.61	7.52	8.38	23.17	23.48	24.28	23.57
	Short	2.04	15.41	46.77	116.77	7.19	6.46	5.74	7.08	24.68	22.10	24.05	24.71
Upper stems	Long	0.71	5.74	15.94	46.47	5.70	5.68	6.24	13.24	19.10	18.00	18.44	13.25
	Short	0.51	2.98	9.06	33.46	6.09	5.42	5.51	9.66	24.07	21.81	18.92	16.14
Middle stems	Long	1.95	20.81	69.93	74.51	5.45	5.43	8.21	14.09	19.01	18.78	16.22	10.33
	Short	0.79	9.39	35.65	55.27	6.18	4.31	5.48	9.47	18.90	24.86	20.54	14.26
Lower stems	Long	1.00	12.29	25.02	73.91	12.76	10.94	16.17	20.21	9.09	10.33	8.79	7.66
	Short	0.86	5.63	21.87	66.14	10.60	8.41	10.22	14.37	11.27	15.17	13.23	11.18
Entire tops	Long	12.73	96.04	255.81	476.00	8.41	8.05	9.57	13.10	18.15	17.92	16.84	13.34
	Short	10.59	62.67	197.05	449.03	7.83	6.42	6.73	9.74	20.31	20.17	19.82	17.26
Entire roots	Long	9.80	20.08	39.05	121.37	4.69	6.34	12.17	14.61	13.12	13.83	17.02	7.90
	Short	6.05	7.88	22.39	110.20	4.72	5.00	10.10	11.42	18.14	16.72	12.75	12.62
Entire plant	Long	22.53	116.12	294.86	598.06	6.80	7.76	9.91	13.40	16.64	17.34	16.87	12.13
	Short	16.64	70.55	219.44	559.23	6.70	6.26	7.10	10.07	19.76	19.87	18.79	16.22

greater than the leaves of the long-day group. Middle and lower leaves (table III) of short-day plants were higher in water and total nitrogen. The average area of middle and lower leaves in short-day exceeded that of corresponding long-day foliage, indicating that more rapid growth would probably have resulted in greater size of all individual leaves in short-day plants had they been grown to maturity.

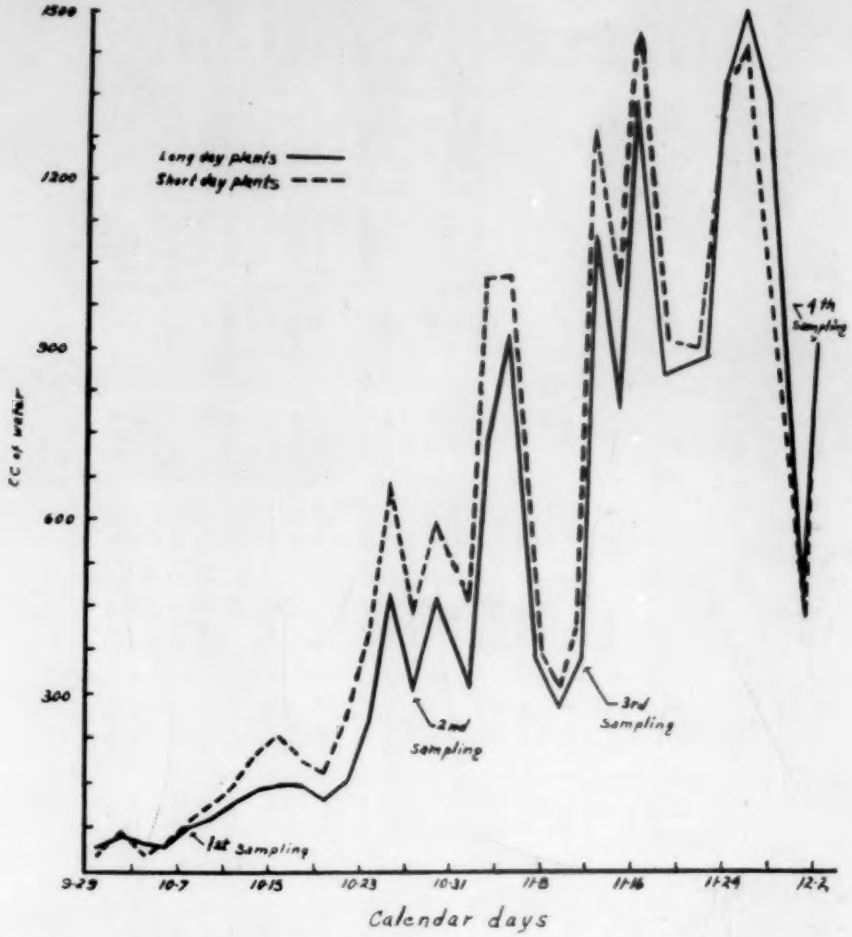

FIG. 3. Water consumption by two-day periods. Age in days: 1st sampling, 24 days; 2nd sampling, 44 days; 3rd sampling, 59 days; 4th sampling, 79 days.

The fresh weight per square centimeter of leaf area was highest in the lower leaves and least in the upper leaves for both groups of plants (table IV). The dry weight data substantiate the previous statement that the long-day group assimilated more food than the short-day group. The dry weight gain of the shoots and roots was much slower in the short-day group during the first half of the experiment but comparable during the latter half

though the short-day group had not yet surpassed the long-day group at final sampling. In terms of growth, the plant in the short-day group remained about 8 days behind the long-day group.

Since a record was kept of the water consumption for each two-day period (fig. 3), it is possible to determine the water demand of the plants at different times of growth directly without reference to the fresh and dry weights. A sharp drop was noticed in the water consumption between November 6 and 12 in the elongation phase of both long- and short-day plants. In view of the continued uniformity of the environment, this drop in water consumption occurring consistently in all plants of both series reflects an altered metabolism which is probably associated with a developmental change (**28**), the exact nature of which cannot be verified without inspection of histological samples. A later report on the histological development of the tissues is contemplated in order to determine the structural changes associated with major shifts in composition. Floral buds were first barely visible by unaided eye in long- and short-day plants between November 8 and 10. This fact suggests some correlation between inception of the reproductive structures and the marked shift in water demand of the plants at that time (**14**). During their entire development the total water consumption of the long-day group of plants was only 2200 cc. more than that of the short-day group.

The sharp drop in the water intake during the flower bud stage was much greater in the short-day group. Up to this date the actual water requirement per gram dry weight increment of plants in this group was nearly 50 per cent. greater than for long-day plants. At all phases of growth the water requirement per gram dry weight gain for the short-day group of plants was higher than for the long-day group. The water requirement of long-day plants at the flower bud stage was the lowest figure in the entire water record.

Except for the stem in the early stage of growth, the short-day plants have a higher percentage water content than the long-day group at all stages and for all portions. Despite the shorter stem length, the water gradient between the upper and lower leaves is greater in the short-day group except at the time of the first sampling (table III). Prior to flowering all parts of the plants in both groups are comparatively low in percentage dry weight (table IV). Reduction in water content is most marked in stems in the final phase. The general trend toward tissue dehydration which is coincident with maximal water absorption of both long- and short-day plants in the flowering phase indicates the plants' inability to balance water intake against outgo at this stage even under conditions of constant moisture supply.

The long-day group of plants formed considerably more total hydrolyzable carbohydrate than the short-day group (tables V and VI). The carbohydrate data suggest that there is difficulty in distribution of these materials in the short-day group. At the time of the last sampling, the leaves of the short-day group contain a much larger amount of carbohydrate

than the leaves of the long-day group, but the stems of the long-day group contain more carbohydrate than the stems of the short-day group. Apparently the long-day group had shifted a large portion of reserve carbohydrates from the leaves to the stem by the time of full anthesis.

The distribution of the carbohydrates at each sampling shows noticeable differences between the two groups in the location of the major fractions of these components (table VII). Long-day tops show progressive loss of

TABLE V

NITROGEN ANALYSES OF PLANTS GROWN IN 14- AND 6½-HOUR DAY LENGTHS. RESULTS GIVEN IN PERCENTAGE OF DRY WEIGHT

SEGMENT	DAY LENGTH	TOTAL NITROGEN				SOLUBLE NITROGEN		
		24 DAYS	44 DAYS	59 DAYS	79 DAYS	44 DAYS	59 DAYS	79 DAYS
		%	%	%	%	%	%	%
Upper leaves	Long	6.20	6.34	6.66	5.16	0.79	0.96	0.74
	Short	5.63	6.14	6.48	7.12	0.80	1.12	1.27
Middle leaves	Long	4.09	5.90	5.76	5.00	0.83	1.25	0.56
	Short	4.92	7.12	6.71	6.43	2.10	2.34	1.29
Lower leaves	Long	1.54	3.44	3.46	7.32	0.61	0.38	0.41
	Short	2.29	5.63	6.19	4.89	1.96	1.98	1.18
Total leaves	Long	4.26	5.35	5.56	5.56	0.75	1.13	0.58
	Short	4.41	6.47	6.50	6.03	1.88	2.02	1.25
Upper stems	Long	3.08	.....	4.81	2.68	1.62	1.60	0.91
	Short	2.08	.....	3.83	3.55	2.16	1.62	1.31
Middle stems	Long	1.92	.....	2.70	1.64	2.10	1.43	0.75
	Short	3.24	.....	4.14	2.77	3.22	2.57	1.28
Lower stems	Long	1.20	.....	1.51	1.15	0.87	0.61	0.53
	Short	5.80	.....	2.55	2.20	1.18	1.32	1.14
Total stems	Long	1.82	2.83	2.45	1.61	1.46	1.14	0.68
	Short	4.68	3.56	3.35	2.61	2.12	1.88	1.21
Total tops	Long	3.64	.....	4.19	3.52	1.01	1.09	0.60
	Short	4.46	.....	5.39	4.63	1.94	1.97	1.18
Total roots	Long	2.63	3.01	3.07	2.46	0.50	0.33	0.22
	Short	2.95	3.45	2.88	2.71	1.00	0.50	0.40
Entire plant	Long	3.33	3.7	4.01	3.29	0.89	0.97	9.52
	Short	4.04	5.5	5.03	4.21	1.86	1.75	1.01

hydrolyzable carbohydrate, involving especially marked depletion of upper leaves with corresponding accumulation in roots and lower stem segments. The changes are steady and progressive with increasing age. The rapid accumulation of carbohydrates in the roots of the long-day group in the flowering stage suggests that roots compete vigorously with the flowers for carbohydrates.

In the short-day group, on the other hand, the loss of foliar carbohydrates is much less marked, showing in fact some accumulation rather than depletion in basal leaves at the time of final analysis. There is, consequently, a smaller fraction of the total carbohydrates in the stems, especially at the flowering stage. Short-day roots contain a smaller fraction of the

TABLE VI

CARBOHYDRATE AS PERCENTAGE DRY WEIGHT OF PLANTS GROWN IN 14- AND 6½-HOUR DAY LENGTHS

SEGMENT	DAY LENGTH	TOTAL CARBOHYDRATE				SOLUBLE SUGAR				POLYSACCHARIDES			
		24 DAYS	44 DAYS	59 DAYS	79 DAYS	24 DAYS	44 DAYS	59 DAYS	79 DAYS	24 DAYS	44 DAYS	59 DAYS	79 DAYS
		%	%	%	%	%	%	%	%	%	%	%	%
Upper leaves	Long	17.05	11.58	7.46	7.50	3.41	3.03	2.37	1.23	13.64	8.55	5.09	6.27
	Short	10.92	7.86	8.48	15.16	1.26	0.85	2.74	2.11	9.66	7.01	5.74	13.05
Middle leaves	Long	16.39	9.09	6.45	9.11	2.73	2.49	1.47	1.46	13.66	6.60	4.98	7.65
	Short	9.89	5.27	7.43	15.27	2.56	0.72	1.15	2.93	7.33	4.55	6.28	12.34
Lower leaves	Long	20.96	8.39	10.36	13.97	6.00	1.81	3.38	3.22	14.96	6.58	6.98	10.75
	Short	10.88	4.32	6.41	21.27	2.04	1.01	1.94	5.09	8.84	4.31	4.47	16.18
Total leaves	Long	17.57	9.49	7.29	9.92	.....	.....	.....	.....	.....	.....	.....	.....
	Short	10.49	5.36	7.29	17.45	.....	.....	.....	.....	.....	.....	.....	.....
Upper stems	Long	27.50	27.91	30.25	20.93	17.50	1.87	22.92	11.26	10.00	11.04	7.33	9.67
	Short	12.90	20.37	24.85	18.10	3.23	8.64	16.03	9.62	9.67	11.73	8.82	8.48
Middle stems	Long	43.40	30.53	14.04	25.92	28.30	20.26	9.04	13.75	15.10	10.27	5.00	12.17
	Short	10.20	15.80	11.92	20.02	4.08	8.15	6.24	10.05	6.12	7.65	5.68	9.97
Lower stems	Long	19.53	18.51	14.09	23.99	8.59	6.10	4.64	5.74	10.94	12.41	9.45	18.25
	Short	20.88	10.15	13.15	17.18	2.20	0.63	1.92	4.84	18.68	9.52	11.59	12.34
Total stems	Long	29.93	24.46	15.54	24.03	.....	.....	.....	.....	.....	.....	.....	.....
	Short	16.37	13.94	14.06	18.18	.....	.....	.....	.....	.....	.....	.....	.....
Entire tops	Long	20.73	14.91	10.93	16.96	7.19	6.28	4.92	5.97	13.54	8.63	6.01	10.99
	Short	11.70	7.58	9.68	17.83	2.17	1.86	2.93	5.38	9.53	5.71	6.75	12.45
Entire roots	Long	18.87	24.51	17.19	27.44	7.38	6.76	2.84	7.28	11.49	17.75	14.35	20.16
	Short	14.69	21.32	12.52	18.45	2.10	2.03	1.24	7.10	12.59	19.29	11.28	11.35
Entire plant	Long	20.17	16.27	11.95	19.28	7.25	6.35	4.58	6.26	12.92	9.95	7.38	13.02
	Short	12.47	8.80	10.09	17.96	2.15	1.88	2.68	5.77	10.32	6.92	7.41	12.19

total amount of carbohydrates in the plants than found in the long-day roots and the root fraction of carbohydrates apparently reaches a critically low level in the flower bud stage. The root carbohydrates at this stage may be low enough to be the limiting factor in root growth. HOAGLAND, STEWARD, BROYER and others (**4, 8, 15, 19, 20, 21, 22**) have shown that salt absorption tends to be impaired by low root respiration resulting from insufficiency of available carbohydrates. The long-day inflorescences despite their large

TABLE VII

PERCENTAGE DISTRIBUTION OF TOTAL SOLUBLE AND HYDROLYZABLE CARBOHYDRATES* AND TOTAL NITROGEN† OF THE PLANT

	Day length	Carbohydrates				Nitrogen			
		Age in days				Age in days			
		24	44	59	79	24	44	59	79
		%	%	%	%	%	%	%	%
Total leaves	Long	45.3	31.9	28.6	14.8	66.7	71.3	65.0	49.5
	Short	49.7	41.1	40.0	39.0	64.5	79.2	71.6	57.8
Upper leaves	Long	14.6	9.1	5.7	3.2	31.4	19.7	15.1	12.9
	Short	18.7	9.5	8.1	8.1	28.0	11.9	12.4	16.3
Middle leaves	Long	19.4	15.2	16.2	6.2	29.4	39.2	43.2	19.9
	Short	19.4	20.6	20.9	13.6	28.9	44.3	38.0	24.4
Lower leaves	Long	11.3	7.6	6.7	5.4	5.9	12.4	6.7	16.7
	Short	11.5	11.0	11.0	17.3	6.7	23.0	1.2	17.1
Inflores-cences	Long	.....	.....	.....	4.6	.....	.....	.....	14.7
	Short	.....	.....	.....	4.8	.....	.....	.....	8.1
Total stems	Long	26.6	46.8	48.0	49.1	9.8	18.4	22.5	19.3
	Short	20.2	37.3	42.0	32.3	17.7	15.1	20.1	19.7
Upper stems	Long	3.6	6.2	8.6	8.3	2.0	2.7	4.1	6.3
	Short	2.9	8.5	7.9	5.8	2.2	2.0	2.4	4.8
Middle stems	Long	14.9	23.6	23.1	17.6	3.9	7.0	13.2	6.5
	Short	3.6	16.5	14.9	10.4	4.4	7.8	10.4	6.1
Lower stems	Long	8.1	17.0	16.3	23.2	3.9	8.7	5.2	6.5
	Short	13.7	12.3	19.2	16.1	11.1	5.3	7.3	8.8
Total tops	Long	71.8	78.7	76.6	63.9	76.5	89.7	87.5	68.8
	Short	69.8	78.4	82.0	71.3	82.2	94.3	91.7	77.5
Total roots	Long	28.2	21.3	23.4	31.5	23.5	10.3	12.5	16.5
	Short	30.2	21.6	18.0	22.9	17.8	5.7	8.3	14.4

* Total weight of soluble and hydrolyzable carbohydrates per plant = 100%.
† Total weight of nitrogen per plant = 100%.

size contain a somewhat smaller proportion of the plant's total carbohydrate content than do the short-day inflorescences. The entire carbohydrate picture in the short-day group is one of relative carbohydrate immobility compared with plants in long day lengths.

There is a marked shift in the ratio of soluble to insoluble carbohydrate between the third (age 59 days) and fourth (age 79 days) samplings in the two series. With increasing age the tops of the long-day group show a general drop in the percentage of soluble carbohydrates while the short-day

group shows a general increase (table VI). The low point for soluble carbohydrate in the roots of both groups occurs at the flower bud stage. During blooming there is a sharp rise in the soluble carbohydrate content of all portions of the plants, the rise being greatest in the case of the short-day roots.

The actual amount of total nitrogen is greater at all stages in the long-day group of plants, but the short-day group has a higher protein percentage on the dry weight basis (table V). In the last stage of growth, the inflorescences tend to accumulate nitrogen at the expense of the foliage in both the long- and short-day groups, despite a simultaneous increase in the root fraction in this late phase (table VII). The nitrogen fraction of both the long- and short-day roots is lowest during the phases of rapid elongation. Leaves of the short-day group possess a greater fraction of the total nitrogen content during the last two stages of growth than do the leaves of the long-day group, suggesting that the movement of nitrogen from the shoot to the inflorescence is much slower in short day. The greater concentration of nitrogen per unit dry weight in short day is associated with a high degree of succulence of entire plants (**13**). Total nitrogen per unit leaf area rises progressively with age in both series, but the advance is more rapid in the long-day group.

The tops of the long-day group contain a greater fraction of total ash for each period than those of the short-day group (table IV). The total ash content of roots tended to rise more rapidly in the long-day group. At the time of the last sampling, however, the short-day roots were higher in total ash content than the long-day roots. The dry weight per unit of ash rises with age in both groups, but much faster in the long-day group (table VII). This rise is especially large in the long-day group between the flower bud and early fruiting stage. The greatest weight of ash per centimeter of leaf area is found in the lower leaves in both groups (table III). At all levels the long-day leaves contain a greater ash weight per unit leaf area than the short-day leaves. At the time of blooming and early fruiting there is apparently a migration of ash from the lower leaves to the upper leaves in the long-day group, but there is no indication at that time of a similar migration of the ash in the short-day plants.

Conclusions

There was a distinct difference in the vegetative vigor of the long- and short-day plants of Little Turkish tobacco. Short day length somewhat prolonged the vegetative phase. The increase in growth is primarily correlated with rate of increment in foliar area. Short day length favors formation of more leaves but the average area per leaf is smaller and the internodes are shorter. The aggregate leaf area of the short-day plants ultimately surpasses that of the long-day plants. This response, coupled with a longer vegetative period of growth, suggests an eventually greater vegetative yield of the short-day plants at maturation.

WEAVER and HIMMEL (**26**), working with a number of photoperiodic plants found that under short-day illumination the growth of both tops and roots was greatly retarded, approximately to the same degree in both long- and short-day species. In the present experiment with tobacco similar results were obtained, except that the development of the roots was retarded to a greater degree than the tops by short day during the rosette and elongation stages. As soon as a certain structural balance between the tops and roots of the plant was attained in the short-day illumination, rapid growth of plants occurred. Had all plants been grown to maturity, the vigor of short-day plants at the last sampling indicated that the final yield of short-day plants might have exceeded that of long-day plants.

The accumulation of carbohydrates in the plants evidently was in a large measure a function of the amount of light received (**6**). Carbohydrates synthesized in the short-day group of plants tended to accumulate in the leaves, showing the relative inability of the short-day plants to translocate carbohydrates. GRAINGER (**7**) observed a comparable response in Chrysanthemum under short-day conditions.

During growth, shifts in water balance are associated with distinct changes in structure and qualitative shifts in metabolism. SMIRNOV (**18**) found that during the blooming period of tobacco the water intake and respiratory energy fell temporarily but increased again after anthesis. Water absorption in the present study parallels that noted by SMIRNOV in showing a drop at the beginning of flowering (fig. 3). The data in the present paper, however, do not coincide with those of SANDE-BAKHUYZEN (**17**) on wheat grown in continuous day which show a sudden rise in the transpiration rate at the time of flowering. There is a slight rise in the rate of water intake at the time of the rapid elongation of the tobacco stem, but a marked temporary drop in the rate of absorption occurs thereafter. There is a sharp acceleration in the rate of water demand of Little Turkish tobacco both in long and short day during the late flowering and early fruiting stage.

Summary

The Little Turkish variety of tobacco grown in 6½- and 14-hour day length, respectively, with constant salt and water supply in gravel culture show that:

1. Stem elongation in the short-day group of plants is retarded in all phases of growth.

2. Internodes are shorter and average area per leaf is smaller in short day, but the number of leaves is greater.

3. Leaf surface area increases at a greater rate in the long-day group of plants until the time of flowering. During flowering, the aggregate foliar surface increases more rapidly in short-day and finally surpasses the total leaf surface of long-day plants.

4. The rate of root growth is greatly retarded in the short-day plants during the stage of rapid stem elongation.

5. The long-day group of plants produce a greater total dry weight yield. Plants of the short-day group remain about eight days behind the long-day group in growth.

6. Total water consumption is similar in the two groups. Because of the smaller size of short-day plants, the amount of water consumed per gram of dry weight of tissue produced (water requirement) is much greater.

7. Water consumption per unit leaf area rises sharply during the blossoming and early fruiting phases in both long and short day, but the rise is about twice as great in the long-day group.

8. Carbohydrates of the short-day group are relatively immobile, tending to accumulate in the leaves.

9. Nitrogen tends to accumulate in stems of both groups at the expense of the leaves in later stages of development.

10. There is a progressive increase in total ash content with age in both long- and short-day groups, but during the final phase of growth short-day plants increase their ash at a greater rate.

11. Inflorescences in long-day are more than twice as large and the size and the number of flowers is greater than in the short-day plants.

12. Formatively, short-day leads to production of more but smaller vegetative elements (leaves and internodes), poorer root growth, and fewer reproductive elements (flowers and fruits).

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TEMPERATURE–GROWTH RELATIONS OF THE ROOTS AND HYPOCOTYLS OF COTTON SEEDLINGS[1]

C. H. Arndt

(WITH SIX FIGURES)

Whether root systems and tops of plants are essentially alike or notably different with regard to their temperature relations is an important question in fundamental plant physiology, as well as in ecology, in pathology, and in the study of the climatic distribution of plants in nature and in cultivation. This general question is dealt with in the present paper with respect to seedlings of the cotton plant, *Gossypium hirsutum* L. The results presented show elongation rates of primary roots and hypocotyls for each of 8 different maintained temperatures, all parts of each seedling being exposed to the same temperature. Data are also included on the development of cotyledons and secondary roots and on seedling weights.

Methods

The experimental methods employed in these studies were similar to those frequently used in the study of seed germination, but arrangements were such as to permit observation and measurement to be continued until the hypocotyls and primary roots might each attain a length as great as 200 mm. and 130 mm., respectively, at the more favorable temperatures. Observations were continued for as long as two weeks at the lower temperatures and for a week at the more favorable temperatures for seedling growth.

The lot of seed used was an upland variety, Farm Relief No. 2, and was obtained from flowers selfed locally in 1938. About 6 weeks after harvest, the air-dried seeds were delinted (by a 10-minute immersion in H_2SO_4), and thoroughly washed in tap water containing an excess of $CaCO_3$; all floaters, or about 20 per cent. of the total amount, were discarded. The rest were air-dried until the moisture content was reduced to 10 per cent. and were then stored in a glass container at laboratory temperature. The dried seeds were found to have a mean weight of 0.112 gm. and to vary in weight from 0.06 to 0.16 gm. By discarding about 60 per cent., a selected stock of seed with individual weights of 0.100 to 0.125 gm. was obtained for the cultures.

For each series of cultures, seeds from the selected stock were surface-sterilized for 90 sec. with $HgCl_2$ (0.25 gm. per liter of 50 per cent. ethanol), after which they were rinsed in sterile tap water, and distributed to the culture tubes. Preliminary tests had shown that this treatment for surface sterilization prevented growth of fungi on the seed coats without affecting time or percentage of germination. Special tests had indicated that the period of immersion in the $HgCl_2$ solution might have been as long as 180

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sec., but the 90-sec. period was employed throughout the main study which was carried out between November, 1939, and April, 1940.

The culture medium used was an agar gel, containing 20 gm. of agar per liter of nutrient solution. The latter was essentially Knop's solution and contained the following salts at the molar concentrations indicated: $Ca(NO_3)_2$, 0.0028 M; KNO_3, 0.0014 M; KH_2PO_4, 0.0007 M; $MgSO_4$, 0.0010 M. The pH value of the gel medium was about 6.5 at the start and it fell to about 5 during the growth of the seedlings.

The culture containers were ordinary test tubes (14 mm. in diameter and 150 mm. high), containing medium to a depth of 130 mm. After the medium was introduced, the tubes were covered with inverted loosely fitting cylindrical glass vials and were then steam sterilized. When the agar had set, each tube received a single seed, pressed into the agar medium at the center of its free surface with a forceps so that the seed was about half embedded with the micropylar end directed downward. After the seeds had been introduced, the covered tubes were immediately placed in the culture chambers. When the elongation of the hypocotyls began to raise the cotyledons above the agar, each vial cover was replaced with an inverted loosely fitting 18×150 mm. test tube, which rested loosely on a rubber band that encircled each culture tube about 3 cm. below its top so as to permit gas exchange between the external atmosphere and the enclosed space above the agar. It was possible for a hypocotyl to attain a length of approximately 125 mm. before the cotyledons reached the top of the cover; but, when that was about to occur, the cover was removed.

The collet, which remained approximately at the surface of the agar gel, was used as a point of reference for daily measurements to the nearest millimeter of the length of each taproot and hypocotyl. Measurements of the taproots were discontinued when they had attained a length of about 125 mm., since the root tips had then about reached the bottom of the culture tubes.

Each taproot grew downward against the wall of its tube, causing the gel to separate from the glass and form a continuous air space from 2 to 5 mm. wide on each flank of the root. Secondary roots, when formed, grew laterally into these air spaces, causing further separation of the gel from the glass wall as far as they extended. The thickness of the lateral air spaces was usually equal to the diameter of the root or roots that produced them; thus, each root lay against glass on one side and agar gel on the opposite side, being generally in contact with air on the flanks. Through the shrinkage of the gel at a temperature of 24° C. and higher, the two air spaces tended ultimately to become united, because the taproot adhered to the agar as it was drawn away from the glass. The shrinkage of the agar facilitated air diffusion downward along the roots, which appeared to be adequately supplied with oxygen, even to a depth of 125–130 mm., when such lengths were attained. The seedlings formed in these test-tube cultures were comparable to seedlings which have grown in sand or solution cultures under the same

conditions of temperature, light, and humidity and for the same culture periods.

At each of the temperatures used, there were regularly 100 single-seed cultures, but most of the data studied were based on the first 50 seeds to germinate. It is appreciated that this procedure resulted in an additional selection, besides the original one based on seed weight. That seedlings for experimentation may be selected according to promptness of origin has been noted by several authors (**11**). Obviously, this second selection could be applied only after germination, and it is naturally impossible to ascertain whether the individual seeds which germinated most promptly at any temperature would have exhibited a similar degree of promptness if they had been germinated at some other temperature. It seems clear, however, that the seedlings from the first 50 seeds to germinate at any temperature were more nearly alike—at least with respect to promptness of origin—than those of the entire group produced at that temperature. At temperatures from 18° to 36°, not less than an additional 40 seedlings were produced within less than 24 hours after the first 50.

To gain some information concerning the relations between promptness of germination and seedling growth, two special experiments were carried out at 27° and 33° C. In each case the seedlings from the first 50 seeds to germinate, those from the second 50, and those from the third 50 were studied as three separate groups for a culture period of 7 days. At these two temperatures, growth was essentially alike for the three groups after 3 to 4 days of culture. It thus appeared that differences related to germination time were effective to influence growth only in those very early stages that constitute germination or immediately follow it. It, therefore, seems evident that the second selection, based on the use of only 50 seedlings from each set of 100 seeds, did adequately represent this stock of seeds.

The eight temperatures used were: 18°, 21°, 24°, 27°, 30°, 33°, 36°, and 39° C., each automatically maintained within a range of $\pm$ 0.25° by means of an electric heater in the lower portion of the growth chambers and, when necessary, by the circulation of cold water through copper tubes in the upper portion of the chambers. The chambers were well-insulated rectangular boxes, 80 cm. wide, 120 cm. long, and 80 cm. high. A small electric fan stirred the air in each chamber, and the air temperature about the upright, centrally-placed cultures was essentially the same at all levels. At all temperatures, a relative humidity of 85–90 per cent. was maintained by means of simple evaporation for the tests at temperatures of 18° to 27° C. and by means of mechanical humidification for the temperatures of 30° to 39°. However, the aerial portions of all seedlings were enclosed under their tube covers until they had reached about the same stage of development (as has been noted); the humidity of the air around the hypocotyls and cotyledons was perhaps as high as 95 per cent. or higher until the covers were removed. It would have been preferable, if the apparatus had been available, to have maintained a uniform water-vapor saturation deficit, since the drying capac-

ity of the air in the absence of wind and light is roughly proportional to this deficit (**14**). At 39°, the deficit was slightly more than 3 times as great as at 18° C. Nevertheless, the transpiration must have been relatively low at all temperatures, since the largest possible vapor deficit, at 85 per cent. relative humidity and at 39° would have been equivalent to 7.87 mm. of mercury.

No light entered the culture chambers, and the seedlings were not exposed to any illumination, except when they were removed from the chambers for about 15 minutes each day for making the regular observations with a 100-watt tungsten electric bulb located about 150 cm. above the seedlings. In no instance was any greening evident.

The regular period of these tests was generally 7 days. Because of the slow growth at 18°, 21°, and 24°, however, two culture sets were simultaneously grown at each of these temperatures. One set was used to furnish the 7-day-weight data for comparison with those obtained at the other temperatures, while the other set was continued for growth observation throughout a period of 10 days at 24° and 14 days at 18° and 21° C.

TABLE I

LENGTHS OF PRIMARY ROOTS AND HYPOCOTYLS FOR EACH TEMPERATURE AT THE TIME OF THE MEASUREMENT WHEN THEIR MEAN LENGTHS WERE NEAREST 100 MM., ALSO CULTURE PERIOD REQUIRED TO REACH THESE LENGTHS AND STANDARD ERRORS OF THESE MEANS AS PER CENT. OF THE MEAN

Temperature	Primary roots			Hypocotyls		
	Culture period	Mean length	Standard error	Culture period	Mean length	Standard error
°C.	*days*	*mm.*	*%*	*days*	*mm.*	*%*
18	14	88.4	1.03	13	98.4	1.12
21	8	97.9	1.87	8	98.0	1.39
24	5	94.4	1.29	5	87.0	1.25
27	4	123.1	2.00	4	93.7	1.15
30	4	93.4	1.79	4	102.2	2.46
33	4	105.7	1.61	3	82.1	0.89
36	4	99.6	1.60	3	71.3	2.23
39	5	47.0*	3.01	7	82.8	1.88

* Less than 35% of the roots continued to elongate after the 5th day.

For the first 50 seedlings that emerged at each temperature, the mean length of primary roots and the mean lengths of hypocotyls were computed for each set of measurements, and these means are shown by graphs (fig. 1). From these means, of which those of table I are typical, were derived the values shown in figures 2 and 3. Statistical analyses showed that the standard error of any means plotted as an ordinate in the graphs of figure 1 was never more than 5 per cent. of the mean itself and for means of 50 mm. or greater was generally less than 2 per cent. The standard errors of table I, which are representative, illustrate the degree of variability that characterized these measurements.

In addition to the daily measurements of the primary roots and cotyledons, records were made at each temperature of the time elapsed (a) be-

fore the beginning of cotyledon emergence, (b) when the cotyledons had emerged in one-half of the seedlings, and (c) before the first appearance of secondary roots. Data on seedling weights at the end of the seventh day of culture for 50 seedlings of one test at each temperature were obtained by gently pulling them from the culture medium so as to avoid injury to the roots. Little or no gel adhered to the roots. Testas were removed if they had not already fallen off. After the fresh weight of each set of 50 seedlings was ascertained, the seedlings were dried to a constant weight at 90° C. to obtain the dry weight. Each set of seedlings was next incinerated in an electric muffle furnace. After cooling, the ash was moistened with hydrogen peroxide and returned to the furnace for 30 minutes; it was then cooled and weighed to obtain the ash weight.

Results and discussion

Temperature relations of seed germination

Records of germination time were not precise in this study, but it was observed (fig. 1A) that discernible protrusion of the primary roots occurred most promptly at 33°, and less promptly at 36°, 27°, 39°, 30°, 24°, 21°, and 18° C. in the order named. After seven days of incubation the germination percentage exceeded 90 for all temperatures, excepting 39°; at that temperature the percentage of germination was 50 after one day and 80 after two days, with no subsequent increase. It is evident that the minimum temperature for any germination at all was well below 18°, while the corresponding maximum was somewhat above 39°. In this connection, it may be noted that Camp and Walker (**3**) failed to obtain germination of cotton seed at 14°; Lehman (**10**) and Ludwig (**12**) have reported some indication of limited root growth at 15° and 12°, respectively; and Haberlandt (**6**) obtained 3 per cent. germination of the related species of cotton, *G. herbaceum* L., at 12° after 19 days of incubation. The reduced germination shown at 39° in these experiments were similar to effects recorded by Camp and Walker (**3**) at 38° and 39°. Both Lehman (**10**) and the last mentioned authors found 40° too high for the germination of cotton seed.

According to unpublished temperature records for 10 years, cotton planting usually occurs in northwestern South Carolina when the mean maximal and minimal soil temperatures at a depth of 5 cm. are about 27° and 12°, respectively; and the absolute maximum may be as high as 33°. Thus the soil temperature to which the newly planted seed is first subjected for at least part of the time is favorable for germination and for the early growth of roots and hypocotyls.

As uniformly high germination percentages of over 90 per cent. were shown in these tests at temperatures from 18° to 36°, it is probable that the employment of alternating temperatures, as suggested by Toole and Drummond (**15**), may not be generally necessary in viability tests of cotton seed. In these tests, at least, satisfactory germination of acid-delinted seed occurred at each of the temperatures used, except the injurious one of 39°.

Minimal and maximal temperatures for elongation in primary roots and hypocotyls

Examination of the graphs (fig. 1) shows that the minimal temperature for growth of both primary roots and hypocotyls was below 18°, and that the maximal temperature was above 39°. At 18°, root elongation could first be measured on the 2nd day of incubation, but measurements of hypocotyls could not be made until the 6th day. After measurable elongation had begun, the mean daily increments at this low temperature fluctuated between 5.6 and 8.7 mm. for primary roots, and between 7.1 and 17.2 mm. for hypocotyls. Aside from this slow elongation, the seedlings at 18° remained apparently healthy throughout 14 days of incubation, and they were still growing on the 14th day. Although 18° is clearly above the minimum for

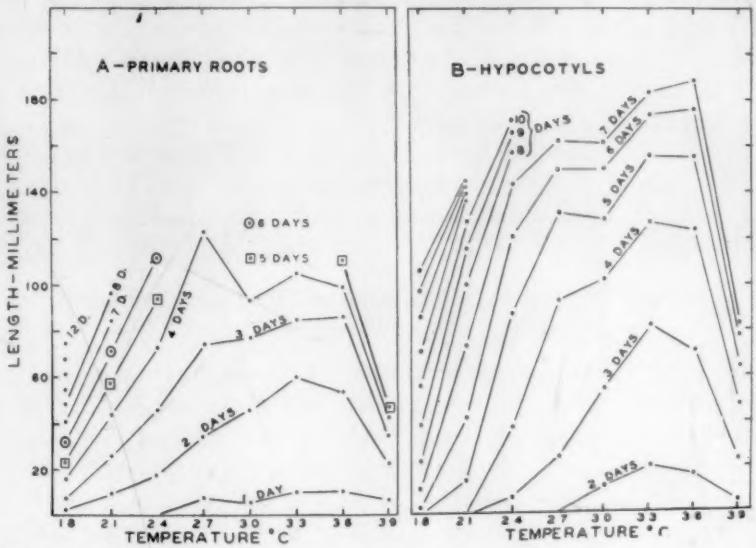

FIG. 1. Mean lengths of primary roots and of hypocotyls after one, two, etc., days of incubation.

some growth of cotton seedlings, the minimum cannot be much lower; FUNG (**4**) did not secure any top growth of cotton seedlings at 15° and ARNDT (**1**) noted discoloration of cotton seedlings growing in soil at 18°. This is also indicated by the failure of the primary roots to form secondary roots during a 14-day incubation at 18°.

At 39° the primary roots had attained a mean length of 7.6 mm. by the end of the first day of incubation, but they showed apparent heat injury and their daily increment fell regularly from 15.5 mm. for the second day to 4.3 mm. for the fifth day. After this time, the increments were negligible, since the length increased only from 47 mm. on the fifth day (table I) to 48.9 mm. on the seventh day. This reduction in growth increments was associated with a comparable reduction in the number of roots which continued to elongate. All of the 50 roots that emerged on the first day of incubation

continued to elongate throughout the first three days, but the number that elongated on subsequent successive days was reduced to 45, 40, 17, and 4. After two days of growth, these heat-injured roots were unusually large in diameter and their tips rounded, although still white. After three days the root tips were generally pointed and yellowish; after five days their color had generally become almost black and all tips were pointed; and they were obviously dead by the seventh day. It is clear that 39°, while submaximal in the sense that it permitted some root growth, was, nevertheless, well above the maximum for sustained healthy development of roots.

At 39°, the hypocotyls could not be measured until the second day, when their mean length was 4.7 mm. For the third day the mean 24-hour increment was 19.1 mm.; for the fourth day, 24.0 mm.; after which, the mean daily increment was progressively less, until it was only 4.9 mm. for the seventh day. As in the case of primary roots, 39° was clearly too high for sustained hypocotyl elongation, although it permitted considerable growth at first in the more resistant seedlings. In the experiments of BERKLEY and BERKLEY (**2**), cotton seedlings were killed at 42° in 48 hours, which indicates that 42° was well above the maximum for growth of roots and tops. FUNG (**4**), however, observed growth of cotton plants at 45° (113° F.) with a relative air humidity of 90 per cent., an observation which is difficult to reconcile with other data.

TEMPERATURE OPTIMA FOR ELONGATION OF PRIMARY ROOTS AND HYPOCOTYLS

Three different procedures were used to estimate the temperature optima for the growth of primary roots and hypocotyls. In the first, it was taken to be that temperature at which the greatest total elongation occurred during a given number of days (fig. 1); in the second, the temperature at which a specified mean length was attained in the shortest time (fig. 2); and in the third, the temperature at which the average growth rate for the attainment of lengths of 50 and 100 mm. was greatest (fig. 3). It is apparent that the derived graphs of figures 2A and 2B resemble in general form the graphs of figures 1A and 1B, respectively, except that those of figure 2 are inverted, the graph maxima in figure 1 corresponding to minima in figure 2.

The calculation of the values used in figures 2 and 3 from the original measurements is based on the supposition that the growth at any temperature proceeded at approximately the same rate through any 24-hour observational period. The derived values are obviously no better than rough approximations, since the measurements show actual changes in the growth rate, as each organ passed through its consecutive growth stages. (Some special tests at 27°, in which the roots and hypocotyls were measured at 9 A.M. and 9 P.M., showed no indication of any day-night periodicity which might have affected the values.) The calculations were carried out only to 0.1 day, since greater precision did not seem to be warranted by the observational data. The actual mean daily elongation was also somewhat greater

than is indicated by the graphs (fig. 3), since the values plotted include premeasurement stages of germination.

The optimum for primary roots was 33° to 36° for the first three days of incubation (fig. 1A), after which it shifted to 27°. That this shift occurred after a length greater than 50 mm., or about 80 mm., is indicated by the graphs of figures 3A, 3B, and 2A. Similarly, an optimum for the hypocotyls of 33° is indicated for the first four days with a tendency for it to shift to 36° after the fifth day of incubation (fig. 1B) and after the attainment of a length of 160 mm. (fig. 2B). This upward shift for hypocotyls is similar to the one found by LEHENBAUER (9) in his experiments with maize shoots, but a downward shift such as that shown here for the primary roots

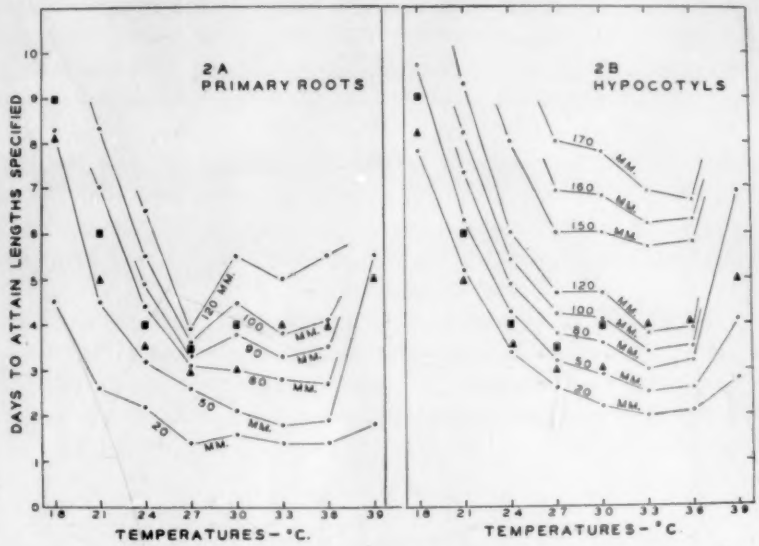

FIG. 2. Approximate number of days required at each temperature for the attainment of the mean lengths indicated; triangles show mean lengths at beginning of the emergence of cotyledons; squares show mean lengths when cotyledons had emerged in half of the seedlings.

seems not to have been noted previously. For roots, the shift occurred at that stage at which the more tardily emerging but more rapidly growing hypocotyls were about equal in length to the more promptly emerging but more slowly growing primary roots.

A double optimum (27°, 33°) is indicated for the primary roots (fig. 1A) for the four-day period. At that time the greatest mean length was shown at 27°; but at 33° and 36° the growth was greater than at 30°, although less than at 27°. Thus, the four-day graph for roots shows a notable depression, or valley, at 30°. In figure 2A a similar relation is shown among these three temperatures after the primary root attained a length of 90 mm., and in figure 3A for the mean growth rate during the period required for it to attain a length of 100 mm. A somewhat similar double optimum seems to be

indicated for hypocotyl growth after five, six, and seven days (fig. 1B). As it seemed possible that the elongation of both organs in these latter stages of growth at 30° might have resulted from accident, a second series of tests was carried out at 27°, 30°, and 33°. Essentially the same results were obtained.

The growth during any 24-hour period showed a similar low elongation for 30°. For the primary roots, the maximal elongation of 49.5 mm. occurred on the second day at 33°; while the maximal elongation at 27° was 47.5 mm. on the fourth day, and at 30° and 36°, 40 and 43.8 mm., respectively, on the second day. For the hypocotyls, the maximal elongation at 33° was 60.7 mm. on the third day; at 36°, 53.3 mm. on the same day; at 27°, 58.9 mm. on the fourth day; and at 30°, 47.2 mm. also on the fourth day.

For the 50 mm. length (fig. 3A), the optimum 24-hour growth rate for both the roots and hypocotyls was 33°, but the rates of root elongation were greater than those of the hypocotyl throughout the temperature range 18°–36°. This was undoubtedly due mainly to the inclusion of unknown time periods before the beginning of measurable elongation. For the 100 mm. length (fig. 3B), the rate of elongation of roots was greater than that of the hypocotyls only at 27°, the root optimum; while, at 30°, 33°, and 36°, the hypocotyl rate exceeded that of the root rate. At the 3 lowest temperatures, the rates for both were essentially the same.

At 24°, 27°, 30°, 33°, and at 36°, the hypocotyls had evidently not yet approached their maximal attainable lengths at the end of the seven-day period of culture, but at these favorable temperatures the graphs indicate that they had passed their period of most rapid growth by the end of the fourth to seventh day. At 21° (fig. 1B), a relatively uniform rate of elongation was maintained for 11 days. The sharply reduced rate after this period seems to indicate that the maximal attainable length of these hypocotyls was less than that of those grown at higher temperatures. At 18°, the reduction in the rate of elongation after the eleventh day was relatively much less than at 21°. Similarly to the hypocotyls, the roots at 18° C. maintained a relatively uniform rate of growth throughout the fourteen-day period of culture. The mean daily elongation rate was 7.17 mm., the maximum was 8.73 mm., and on the fourteenth day was still 7.3 mm. At all higher temperatures, the graphs of figure 1A indicate a decreased elongation after the second or third day of measurable length, except at 27° at which temperature the elongation was 47.5 mm. on the fourth day, or 7.3 mm. greater than on the third day.

Summary and comment on optimal temperatures for growth of primary roots and hypocotyls

The optimum of 27° found for the roots in this study is just slightly higher than the 25° temperature at which Gladys C. Galligar (**5**) secured the most rapid elongation of the excised roots of a closely related species, *G. barbadense*. In table II, interesting comparisons can be made between

the temperature relations of cotton seedlings with those of dark-grown maize and wheat shoots as brought out by the studies of LEHENBAUER (**9**) and of MACK (**13**). The temperature minima for the other two plants is well below that for cotton, and the optimum for wheat is well below that for cotton. The maximum for all three may be about alike, although the highest temperature used for wheat was 30°. A further comparison of the relative growth rates of cotton hypocotyls in these tests and of maize shoots for 12-hour growth periods (**9**) shows relatively the same temperature relations through the temperature range of 18° to 24°.

It is notable that none of the three procedures employed showed 30° as within the indicated optimal range for elongation of either primary roots or hypocotyls. Indeed, it appeared clear that this temperature was relatively unfavorable for these organs after about three days of culture (figs. 1, 2, 3). Whether this feature may have important physiological significance can not be judged without further study, especially planned for that purpose. It

TABLE II

MINIMAL, OPTIMAL, AND MAXIMAL TEMPERATURES FOR COTTON, MAIZE, AND WHEAT SEEDLINGS

PLANT	TEMP. MINIMUM	TEMP. OPTIMUM	TEMP. MAXIMUM
	°C.	°C.	°C.
Maize shoots (**9**)	Below 12	32	Above 42
Wheat shoots (**13**)	Below 10	20–25	Above 30
Cotton hypocotyls 100-mm.	Below 18	33, shifting toward 36	Above 39

may be reasoned, however, that when a temperature optimum for growth shifts with the lapse of time from below 30° to a higher temperature, or vice versa, there might possibly be some intervening interval in which 30° might become the temporary optimal. It does seem likely, however, that when any shift of optimum occurs with lapse of time, the transition should be marked by the occurrence of double optima, such as are shown by the several graphs, at some intermediate time or developmental stage. KOEPPEN (**8**) obtained irregular growth curves for the shoots of *Zea mays* which indicated the possibility of a double optimum of 30° and 33.5° for this plant, but LEHENBAUER (**9**) found no such irregularities. As remarked by HAASIS (**7**), the failure of a set of experimental data to show a double temperature optimum for any length of culture period does not preclude the possibility that a double optimum might become evident with further experimentation, emphasizing other intervals of time and temperature. Thus, if promptness of germination, mean length of primary roots after one day of culture, or mean lengths of hypocotyls after three days were used as criteria in the present study, then 39° would appear to be about as favorable as 27°, 30°, 33°, or 36° for seed germination and early growth of primary roots, and about as favorable as 27° for early growth of hypocotyls; but, as already noted, subsequent effects of heat injury rendered 39° very unfavorable for later growth.

The differences between the temperature relations of roots and hypocotyls appear to be related to the temperatures at which each develops under field conditions. As previously noted, the time of cotton planting generally coincides with the appearance of soil temperatures which are, in part, favorable for rapid growth; while the shift of the root optimum corresponds to penetration of the roots into the deeper and slightly cooler portions of the soil. On the other hand, the hypocotyls, as they elongate, are successively exposed to the mean temperatures of the more shallow portions of the soil and air temperatures which at this time are generally higher, especially during periods of sunshine, than those of the soil at depths of 10 cm. or greater. It thus appears that the difference between root optimum (27°) and hypocotyl optimum (33° or higher) of cotton seedlings may have considerable ecological significance for the cotton plant. It must be borne in mind, however, that these deductions are based on cultures in which the roots and hypocotyls were subjected to the same temperatures, high air humidity, and no illumination; while field conditions involve fluctuating temperatures, differences between air and soil temperatures, greater ranges of air humidity, and daily periods of sunlight.

Temperature relations of secondary roots

Although the temperature growth relations of secondary roots were studied less thoroughly than those of primary roots and hypocotyls, the results of some observations on secondaries may be of interest. No secondaries appeared on the seedlings grown at 39° C. because the primary roots were severely injured or killed before attaining the developmental stage at which they might form branches. Nor did any secondaries appear on the seedlings grown at 18°, although these were observed for 14 days. At 21°, secondaries began to appear only after about ten days of incubation, at 24° and 27°, after six and five days, respectively; and at 30°, 33°, and 36°, after four days of incubation.

At the end of the seven-day incubation period, 12 representative seedlings were selected by simple inspection to supply data on the relative development of the secondary roots. The mean number of secondaries per seedling at 24°, 27°, 30°, 33°, and 36° was approximately 18, 24, 22, 20, and 12; the corresponding mean length of secondaries was about 12, 18, 25, 25, and 28 mm.; and the corresponding mean total length of secondaries per seedling was 216, 432, 550, 500, and 336 mm., respectively.

From these observations, several different temperature optima or optimal ranges are indicated, as follows: for early emergence of secondaries, 30° to 36°; for most numerous secondaries after seven days, 27°; for greatest average length of secondaries after seven days, 36°; for greatest total length of all secondaries present after seven days, 30°. At 27°, 30°, 33°, and 36° most of the secondaries emerged within 48 hours after the first ones appeared; but at 21° and 24° the number of secondaries increased continually from the time when they began to emerge until the end of the observa-

tion period. Consequently, the process of emergence of secondary roots may have temperature relations different from those that affect their subsequent growth. For each temperature at which secondaries developed, the following tabulation (table III) shows the length of primary roots and of hypocotyls at the end of the day when secondaries began to emerge. These lengths are indicated by the ordinates of the circles in figure 5. Mean lengths of both primary roots and hypocotyls at emergence of first secondary roots were greatest at 27°, were least at 30°, and had intermediate values at the other temperatures. When secondary roots began to emerge at 27° and 30° (after five and four days of incubation), 27° was the optimal temperature for elongation of primary roots; but at this time 33° was still the optimum for hypocotyl growth and 30° was clearly unfavorable for both hypocotyls and primary roots.

TABLE III

APPROXIMATE INCUBATION TIME REQUIRED FOR THE APPEARANCE OF SECONDARY ROOTS AND HYPOCOTYLS WITH CORRESPONDING LENGTHS OF PRIMARY ROOTS AND HYPOCOTYLS

TEMP.	TIME FIRST APPEARANCE OF SECONDARY ROOTS	MEAN LENGTH OF PRIMARY ROOTS	MEAN LENGTH OF HYPOCOTYLS
°C.	*days*	*mm.*	*mm.*
18	14+	.....	.....
21	10	98	128
24	6	111	121
27	5	123	132
30	4	93	102
33	4	106	128
36	4	100	125

TEMPERATURE RELATIONS OF EMERGENCE OF COTYLEDONS

The cotyledons emerged earliest at 27° and 30° C. on the third day of incubation (fig. 4). The cotyledons of half of the seedlings emerged first (in three days) at 27°; and slightly less promptly (in four days) at 24° and 30°. The temperature of 21° was clearly suboptimal for prompt cotyledon emergence, and 18° was much more so. The long incubation period (eight days) required at 18° and the failure of CAMP and WALKER (**3**) to obtain emergence of cotton seedlings in soil cultures at 14° should place the minimal temperature for the emergence of the cotyledons at about 15°–16° C.

Temperatures of 33° and 36° were about equally superoptimal for early emergence of cotyledons. A four-day period, which sufficed for emergence in half of the seedlings at 24° or 30°, brought out only a very few cotyledons at 33° or 36°; at the end of a seven-day period only 40 per cent. of the seedlings at 33° and only 20 per cent. of those at 36° had freed their cotyledons, indicating that 36° was by far the less favorable of these two superoptimal temperatures for early emergence. There was, however, no evidence of injury to cotyledons at either of these two temperatures.

At 39°, on the other hand, cotyledon emergence began on the fifth day but all cotyledons that emerged at 39° were already injured, being brownish

in color and obviously unhealthy. At this high temperature, a seven-day period of incubation induced complete emergence in only 4 per cent. of the seedlings, although an additional 22 per cent. showed partially emerged cotyledons at the end of the seventh day, when all cotyledons and hypocotyls had already become brown and so dried as to be evidently dead.

In a special experiment, 20 seedlings grown at 39°, whose primary roots were 10 to 25 mm. long at the end of the two-day period of incubation but

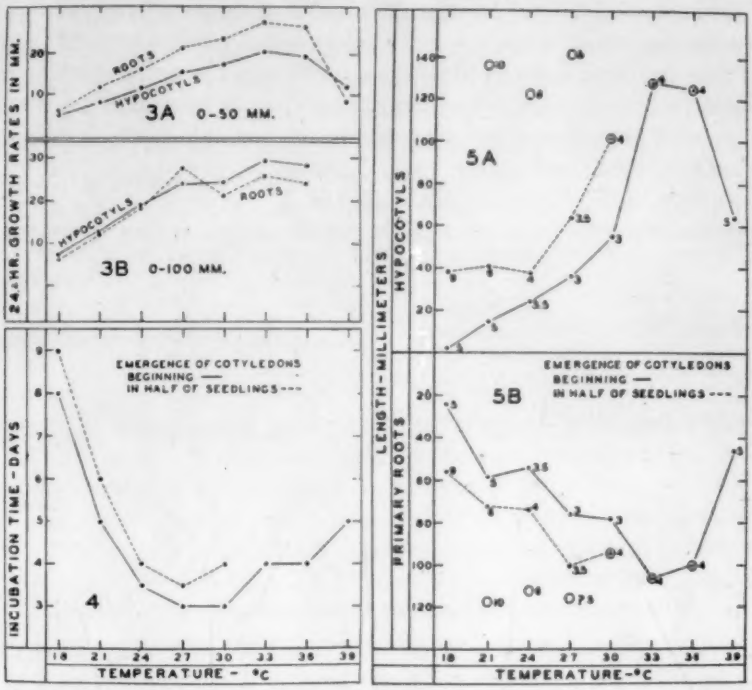

FIG. 3. Average rates of elongation of primary roots and of hypocotyls, while attaining mean lengths of 50 mm. and 100 mm. at each temperature indicated.

FIG. 4. Approximate incubation time elapsed at each temperature indicated before cotyledons began to emerge from testas and when they had emerged in half of the seedlings.

FIG. 5. Mean lengths of primary roots and of hypocotyls at each of the temperatures indicated when cotyledons began to emerge from testas and when they had emerged in half of the seedlings; inscribed numerals indicate the number of days required in each case. Circles indicate mean lengths when secondary roots began to appear and the associated numerals the number of days required.

had failed to elongate further on the third day, were transferred to 25° at the end of that day. Fourteen made no additional growth, but six developed normally. Thus, about 30 per cent. of the seedlings formed at 39° were able to recover from the injurious effects of a three-day period at that temperature when a favorable temperature was provided from the third day onward. On the other hand, 50 seedlings transferred to 25° at the end of a five-day period of incubation at 39° failed to show any subsequent root growth or cotyledon emergence, although a few of their hypocotyls elongated slightly

after the transfer. Thus, a three-day incubation at 39° resulted in the death of about 70 per cent. of these seedlings, and after a five-day incubation at that temperature none of them were capable of recovery.

At 21°, 24°, 27°, and 30° C., the cotyledons also began to emerge when the hypocotyls were shorter than the primary roots (fig. 5); while the reverse was true at the three definitely superoptimal temperatures for emergence, 33°, 36°, and 39°. The lengths of the primary roots and hypocotyls, however, were not the same at the inception of emergence for all temperatures. They increased progressively with the temperature from 21° to 33°, but at 36° they were not greatly different from those at 33°. The few seedlings that brought out their cotyledons at 39° did so when primary roots and hypocotyls were shorter than at 33° and 36°, although the incubation time required for earliest emergence was five days at 39° and only four days at 33° and 36°. It is interesting to note that at 24° and 27°, the secondary roots appeared about 2.5 days after the beginning of cotyledon emergence; while at 30°, 33°, and 36° both secondary roots and cotyledons began to emerge at the same time (fig. 5B).

The relatively low rate of elongation of roots and hypocotyls at 24° seems to indicate why this temperature was too low for the most rapid cotyledon emergence. But why 33° was too high for the emergence of more than 40 per cent. of the cotyledons is not so easily understood, since it was three- to four-day optimum for elongation of primary roots and hypocotyls at this temperature. BERKLEY and BERKLEY (**2**) suggested that the cotyledons of field-grown cotton seedlings may be protected to some extent from heat injury when air temperature is superoptimal but air humidity is low through the cooling effect of relatively rapid transpiration. It is possible that the apparent difference between the temperature relations of cotyledons and those of hypocotyls may have been related in part to conditions of air moisture and absence of light as these influence transpiration. As has been noted, although relative humidity was the same in all culture chambers, the drying influence of the air and, consequently, its cooling effect was greater as the temperature was higher. It seems, however, that cooling through transpiration cannot have been very considerable in these experiments; nor is it likely that it can be very effective under any condition before the cotyledons are well expanded.

TEMPERATURE RELATIONS OF CHANGES IN WEIGHTS AND COMPOSITIONS OF SEEDLINGS DURING THE SEVEN-DAY PERIOD OF CULTURE

As bases for computing weight changes in each set of the 50 seedlings for the seven-day incubation period, the following values were ascertained for a 50-seed sample of the original stock, after the testas had been removed: dry weight, 3.352 gm.; ash content, 0.154 gm.; content of organic matter, 3.198 gm.; ash per gram of dry weight, 0.024 gm. Organic matter means simply the difference between dry weight and ash content. The general smoothness and consistency of the graphs of figure 6 make it apparent that

all these data possess at least a fair degree of reliability with respect to the characteristics of the cotton seed used.

The graphs of figure 6A (also table IV) show that the most favorable temperature for the production of both fresh weight and water content was 30° and that 27°, 33°, and 36° were nearly as favorable. On the basis of elongation (fig. 1), the five-day optimum (and presumably the seven-day optimum) for primary roots was 27°, and the seven-day optimum for hypocotyls was 36°, with 33° only slightly suboptimal; while 30° was relatively unfavorable for the elongation of both organs. These differences illustrate

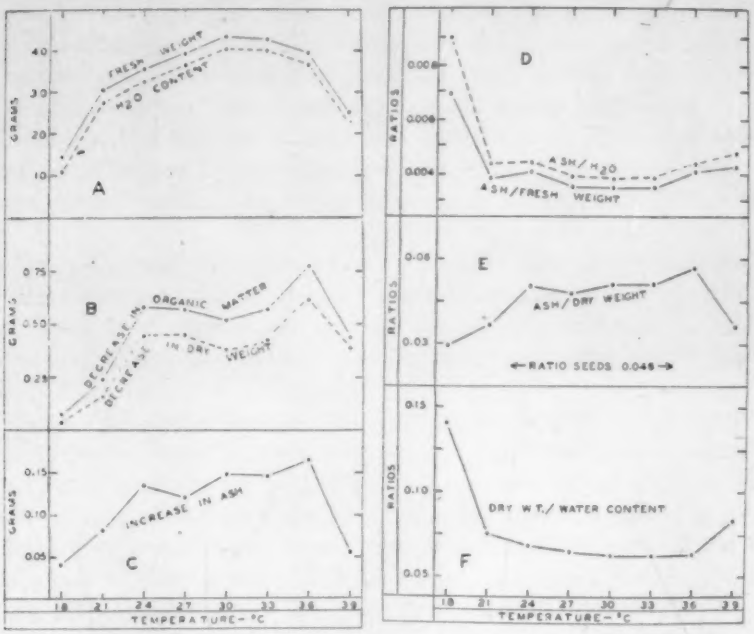

FIG. 6. Weight and composition of lots of 50 seedlings after growth for seven days at the temperatures indicated; also ratios between certain of these values. Allowance was made for the organic matter, dry weight, and ash in the original seeds in calculating the decreases or increases in these constituents during the growth period.

how an optimal temperature based on one criterion of growth or performance (such as fresh weight) may be considerably different from one based on another criterion (such as length). The vertical distances between the two graphs of figure 6A represent the final dry weights of the seedlings. The two graphs of figure 6B have the same general form and show the greatest losses for organic matter and dry weight at 36°. Since both were due to metabolic destruction of organic matter, 36° was apparently most favorable for that process.

Other criteria for estimating the optimal temperature for growth during the seven-day period may be obtained by computing, as in table IV, (a) the mean elongation of the hypocotyl and (b) the water content per unit loss of organic matter at each temperature. The second criterion should be espe-

cially applicable since growth in etiolated seedlings is largely accounted for by water absorption. In addition, special validity to the use of water content as a measure of growth was indicated by the comparable ratio values obtained when the final fresh weight was used in similar computations. According to the above criteria, the optimum would be that temperature at which the greatest elongation of the hypocotyl or greatest water content is associated with the least loss of organic matter. Very different optima are indicated by the two criteria. Thus, 18° was the most favorable for water absorption but was the least favorable for elongation. For the five temperatures favorable for rapid elongation of the hypocotyl, or those from 24° to 36° C., the greatest elongation of the hypocotyl per unit loss of organic matter occurred at 33°, and the greatest water-content per unit loss of organic matter occurred at 30°; but the difference between these two temperatures was small, and they were also the temperatures at which the great-

TABLE IV

LOSS IN ORGANIC MATTER DURING THE SEVEN-DAY GROWTH PERIOD OF SEEDLINGS AS RELATED TO TEMPERATURE, LENGTH OF HYPOCOTYL, AND WATER CONTENT

TEMP.	LOSS IN ORGANIC MATTER	LENGTH OF HYPOCOTYL		WATER CONTENT	
		MEAN	PER MG. LOSS ORGANIC MATTER	PER SEEDLING	PER MG. LOSS ORGANIC MATTER
°C.	mg.	mm.	mm.	mg.	mg.
18	1.36	11	8.1	218	161
21	4.64	74	15.9	548	118
24	11.77	143	12.1	649	55
27	11.53	162	14.1	727	64
30	10.47	162	15.5	810	77
33	11.48	183	15.9	795	69
36	15.69	190	12.1	730	46

est fresh weight and water content were produced. Although the greatest mean elongation of the hypocotyl and the greatest loss in organic matter occurred at 36°, still by both of these criteria this was the least favorable of the temperatures above 24°. Since at 30° and 33° the amount of water absorbed and the elongation of the hypocotyl per unit loss of organic matter were greater than those at 24°, 27°, and 36°, it might be concluded that among these temperatures, organic matter was most efficiently conserved, or effectively consumed, at those two temperatures.

The vertical distances between the graphs for decrease in dry weight and for decrease in organic matter (fig. 6B) represent the corresponding net increases in ash during the seven-day period (fig. 6C). The most favorable temperature was 36°, which was also the most favorable temperature for loss in dry weight and in organic matter, as well as for seven-day elongation of hypocotyls; but 36° was clearly too high for most efficient usage of organic matter, as has been indicated. The graph for increase in the proportion of ash to dry weight (fig. 6E) has a general form almost like those

for gain in ash weight (fig. 6C), for loss in dry weight (fig. 6B), and for loss in organic matter (fig. 6B). The most favorable temperature for all of these was 36°.

On the other hand, at the end of the seven-day period the ash content per gram of fresh weight and per gram of water content (fig. 6D), as well as dry weight per gram of water content (fig. 6F), were relatively small and about alike at temperatures from 21° to 33°. It is to be noted that within that range of temperature at which there was relatively rapid growth, the differences were comparatively small.

SUMMARY OF OPTIMAL TEMPERATURES ACCORDING TO THE SEVERAL CRITERIA EMPLOYED

Seed germination	33°–36° C.
Elongation of primary roots according to	
Mean lengths at daily intervals (fig. 1A)	33°–36° for 3 days, then 27°
Time required for attainment of specified mean lengths (fig. 2A)	33°–36° to about 80 mm., then 27°
Average 24-hour rate for attainment of mean length of 50 mm. (fig. 3A)	33°
Average 24-hour rate for attainment of mean length of 100 mm. (fig. 3B)	27°
Elongation of hypocotyls according to	
Mean lengths at daily intervals (fig. 1B)	33° for 4–5 days, then 36°
Time required for attainment of specified mean lengths (fig. 2B)	33°–36° to about 160 mm., then 36°
Average 24-hour rate for attainment of mean lengths of 50 and 100 mm. (fig. 3A, 3B)	33°
Development of secondary roots according to	
Promptness of emergence (fig. 5)	30°–36°
Number present after seven days of incubation	27°
Average length after seven days of incubation	36°
Total length after seven days of incubation	30°
Promptness of emergence of cotyledons according to	
Earliest emergence (fig. 4)	27°–30°
Emergence in half of seedlings (fig. 4)	27°
Weight data	
Weights at end of seven-day period	
Greatest fresh weight of 50 seedlings (fig. 6A)	30°
Greatest water content of 50 seedlings (fig. 6A)	30°
Weight changes during seven-day period	
Greatest decrease in dry weight (fig. 6B)	36°

Greatest decrease in organic matter (fig. 6B)	36°
Greatest mean gain in hypocotyl length per unit loss in organic matter	21°–33°
Greatest mean water absorption per unit loss in organic matter (24°–36° C. temperature range)	30°
Greatest net increase in ash content (fig. 6C)	36°
Final proportion of components after seven days of culture	
Least ash per gram of water content (fig. 6D)	27°–33°
Least ash per gram of fresh weight (fig. 6D)	27°–33°
Greatest ash content per gram of dry weight (fig. 6E)	36°
Least dry weight per gram of water content (fig. 6F)	27°–36°

General summary

Cotton seedlings were grown from acid-delinted seed in darkness on an agar medium in test tubes at temperatures of 18°, 21°, 24°, 27°, 30°, 33°, 36°, and 39° C. Daily measurements of the lengths of primary roots and hypocotyls were made, and mean lengths for each organ were computed to show the elongation on successive days of incubation. Measurements of primary roots had to be discontinued generally after five to six days, but hypocotyls were measured throughout a seven-day period at each temperature. Data were obtained on time of emergence and development of secondary roots at each temperature, and also on emergence of cotyledons from the testas. From the final fresh, dry, and ash weights and from the corresponding weights of 50 ungerminated embryos, the following values were computed for each temperature: loss in dry weight and in organic matter; net gain in ash content; and the ratios of ash to fresh weight, ash to dry weight, ash to water content, and dry weight to water content. In all cases, roots and tops were at the same specified temperature; but the roots were in an agar medium, while the tops were in air having a relative humidity of 85–95 per cent.

A germination percentage greater than 90 was obtained within seven days at all temperatures, except 39° C. It was apparent, from the lengths of the primary roots after two days of incubation, that discernible protrusion of the radicles occurred most promptly at 33°, less and less promptly at 36°, 27°, 39°, 30°, 24°, 21°, and 18°. At 39°, the germination percentage was 50 after one day of incubation, and 80 after two days, following which there was no further increase. The optimal temperature for prompt germination was apparently within the range 33°–36° C.; the minimal temperature for any germination was below 18°, probably about 16°–17° C.; the maximal temperature for any germination was somewhat above 39° C.

The temperature minimum for growth of either primary roots or hypocotyls was clearly below 18°, and the maximum for any growth was above

39°, but the latter temperature soon induced visible heat injury. For each temperature, the data on elongation were graphed to show: (a) mean lengths attained in successive days of incubation, and (b) mean incubation periods required for the attainment of each of several specified mean lengths. Both methods of treatment showed that the optimal temperature for elongation of primary roots was 33°–36° throughout the first few days of their growth, and that they then shifted downward to about 27°; the optimum for hypocotyl elongation shifted in the opposite direction, after four to five days of growth, from 33° to 36°. For primary roots, a mean length of 50 mm. was attained soonest at 27°, but almost as soon at 33°. For hypocotyls, 33° and 36° were apparently about equally favorable for the early attainment of both these mean lengths, for the shift of optimum from about 33° to about 36° did not occur until these organs showed a mean length of about 160 mm.

The earliest emergence of secondary roots occurred at 33° and 36°, but after seven days of culture their total length was greatest at 30°, and they were most numerous at 27°.

The optimal temperature for early emergence of the cotyledons from the testas was about 27°. After seven days of culture, only about 40 per cent. of the seedlings at 33° had freed their cotyledons; only 20 per cent. of those at 36°, and none of those at 39°. This latter temperature was injurious to all parts of the seedlings after the first few days of culture.

After the first three or four days of culture, growth at 30° was relatively slower than would be expected from the corresponding rates shown at 27° and 33°. Despite this peculiarity, 30° was favorable for the development of secondary roots and cotyledons, and induced the greatest fresh weight and the greatest water content in seven days of culture, although 27°, 33°, and 36° were only slightly less favorable according to these two criteria.

In the seven-day period, dry weight and organic matter decreased and ash content increased at all temperatures, the loss being greatest at 36°; but, with respect to both the seven-day elongation of hypocotyls and increase in water content at the temperatures from 24° to 36°, organic matter was most efficiently consumed at 30° and 33°. After the seven days of incubation, the least ash content per unit of water content and per unit of fresh weight, and also the least dry weight per unit of water content were about equal at 27°, 30°, and 33°; and the greatest ash content per unit of dry weight was found in seedlings grown at 36°.

The results of this study indicate three striking features of temperature relations that seem not to have been recorded hitherto for seedlings of any species:

(1). The temperature optimum for elongation of both the primary roots and the hypocotyls shifted with time and growth. For elongation of the primary roots, the shift was downward from 33°–36° at the start, to about 27° after three to four days of growth; for elongation of hypocotyls the shift was in the opposite direction, but was not as great, being from about 33° at the start to about 36° after about four to five days of growth.

(2). Although the optimal temperatures for elongation of the primary roots and of the hypocotyls were nearly the same (about 33°) for a short time after germination began, they differed by as much as 9° when the primary roots had extended downward about 100 mm. and the hypocotyls had extended upward about 160 mm., or when the two main regions of growth were about 26 cm. apart. If this observation should be found to apply to cotton seedlings in the field, it would indicate that young cotton plants may be remarkably well adapted to the temperature differences between air and various depths of soil by virtue of well-timed shifts in the temperature optima for elongation of primary roots and of tops.

(3). According to the several criteria, the temperature of 30° was favorable for increase in weight and for the development of cotyledons and secondary roots, but proved to be relatively less favorable for the most rapid elongation of the hypocotyls and primary roots of these cotton seedlings when they were about a week old. This characteristic may represent some important physiological feature worthy of special study.

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EFFECT OF CARBON DIOXIDE ON ABSORPTION OF WATER AND NUTRIENTS BY ROOTS[1]

H. T. Chang and W. E. Loomis

The importance of soil aeration to plant development has been recognized since the beginnings of agriculture. As our knowledge of the physiology of roots has grown, the beneficial effects of soil aeration have been assigned to a stimulation of root growth (**4**) and thus an increase of root area and penetration, and more recently to a direct stimulation of aerobic processes of salt accumulation. Hoagland and his associates particularly have shown that the rate of mineral accumulation in roots containing respirable carbohydrates may be positively correlated with the oxygen supply (**8, 24**).

Although many data upon the toxic effect of carbon dioxide on protoplasm are available, this phase of the soil aeration problem has been generally overlooked. Free (**7**) and Kramer (**16**) have shown a reduction of water absorption by roots with increased CO_2 concentrations. Hoagland and Broyer (**9**), however, thought the effect of CO_2 related to its displacement of oxygen, and Arrington and Shive (**3**) concluded that reduced CO_2 concentrations were not a factor in the beneficial effects of the aeration of a modified Knop solution. Many other reports of the beneficial effects of the aeration of solutions and soils have stressed the importance of O_2 and ignored or minimized the rôle of CO_2.

The studies reported here were planned to test the toxicity of CO_2 accumulation as contrasted to an O_2 deficiency, and to study the effect of CO_2 upon the absorption of water and the more important nutrients.

Methods

Seedlings of wheat (*Triticum aestivum*, var. Thatcher), maize (*Zea mays indentata*, var. U.S. 35), and rice (*Oryza sativa*, var. Blue Rose) were started in quartz and grown in a four-salt solution, roughly that given by Loomis and Shull (**18**) modified by reducing the phosphorus concentration by half. The plants were grown in quart jars, three plants per culture, until 5 to 7 weeks old, with solution changes at weekly intervals and additions of iron in ferric citrate as required. Enough plants were started that six cultures of well grown, uniform plants were available for each treatment, except in the rice experiment where lots of eight cultures per aeration treatment were divided between two pH levels.

Solutions were changed two days before an experimental run so that the plants would not be in a state of mineral starvation. Fresh solutions of accurately known composition were then weighed into the treatment jars, gas treatments started, and the plants transferred and left for two days and one night (36 hrs.). Air, CO_2, or N_2 was bubbled briskly through the

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treated lots for ten minutes out of every hour throughout the period. Nitrogen cylinders were not available for the experiments with maize, and a 5-mm. layer of paraffin oil was poured over one set of solutions after the plants were inserted to reduce both oxygen absorption and carbon dioxide loss by the solutions. In the rice experiments half of the solutions in each treatment were brought to an initial pH of 4.0 with H_2SO_4 and held at this point with further additions of acid as required. This treatment was intended to differentiate between the directly toxic effects of CO_2 and its effect on the pH of the solution. Cultures treated with CO_2 alone remained near pH 4.0 throughout the period, even though the bottles were not tightly stoppered to prevent loss of CO_2 between treatments.

At the end of the test periods the jars were reweighed to obtain water losses, and samples were taken for determination of total solids, nitrogen, phosphorus, calcium, magnesium, and potassium. Analyses were corrected for water losses and absorption calculated as milliliters of water or milligrams of element per culture. Analytical methods for nitrogen, calcium, and magnesium were those given by LOOMIS and SHULL (**18**). Potassium was determined by the ceric sulphate method and phosphorus by the stannous chloride, colorimetric method.

Five experimental runs were made: the first with wheat on November 13 and 14, 1942; the second with wheat on January 1 and 2, 1943; the third with maize on February 17 and 18, 1943; the fourth with maize on March 6 and 7, 1943, and the fifth with rice on August 24 and 25, 1943. Evaporation conditions varied, being poorest for the third experiment with maize and best for the August experiments with rice. The original solutions varied also, but were always made up in one lot sufficient for the experiment and analyzed in triplicate with the residual solutions to obtain their exact composition. When absorption was low, as of phosphorus and calcium in some of the experiments, its determination by difference of the content of the solutions before and after absorption by the plants was not fully satisfactory because of the accumulation of experimental errors. The physiological condition of the plants probably varied also in spite of uniform handling, for the variation in some of the series was wide and the responses of the three species differed in some respects.

Results

The general picture given by the data in tables I, II, and III, however, is clear cut in indicating that CO_2 was the dominant factor affecting nutrient absorption under the conditions of the experiments and the most important treatment factor affecting water absorption. Standard errors are given for all of the averages in the tables so that the statistical significance of any pair of determinations can be estimated at a glance. In general the addition of CO_2 significantly reduced the absorption of every substance tested. Bubbling commercial nitrogen through wheat cultures (table I) had no effect. It is not assumed that these cultures were O_2 free, but only that O_2 should

TABLE I

THE ABSORPTION OF WATER AND MINERALS BY WHEAT PLANTS RECEIVING DIFFERENT TREATMENTS

SUBSTANCE ABSORBED	CONTROL	BUBBLED WITH			COMPOSITION OF ORIGINAL SOLUTION
		AIR	NITROGEN	CO_2	
EXPERIMENT I. NOVEMBER, 1942					
Water (ml.)	62.4 ± 2.8	70.0 ± 1.5	.....	40.6 ± 4.6	800.0
Total salts (mg.)	84.9 ± 15.8	117.3 ± 10.6	.....	25.7 ± 11.1	828.8
N	11.1 ± 0.3	17.9 ± 0.3	.....	2.1 ± 1.2	44.3
Ca	3.1 ± 0.9	1.4 ± 0.5	.....	0.02 ± 0.4	78.5
K	7.4 ± 1.5	19.0 ± 2.2	.....	*−2.2 ± 1.4*	129.6
EXPERIMENT II. JANUARY, 1943					
Water (ml.)	53.5 ± 4.0	56.7 ± 2.3	60.4 ± 3.7	39.5 ± 2.9	800.0
Total salts (mg.)	184.5 ± 4.9	185.4 ± 4.1	183.9 ± 3.5	150.3 ± 3.9	1132.5
N	18.0 ± 0.3	17.6 ± 0.3	17.1 ± 0.7	15.1 ± 0.4	64.8
P	2.5 ± 0.2	2.6 ± 0.2	2.1 ± 0.2	1.6 ± 0.5	16.2
Ca	10.3 ± 0.6	9.8 ± 1.5	10.6 ± 0.3	8.2 ± 1.0	95.8
Mg	7.0 ± 0.6	7.4 ± 0.4	7.3 ± 0.6	3.9 ± 0.5	52.2
K	24.0 ± 1.4	27.8 ± 2.0	25.5 ± 0.9	8.1 ± 2.2	135.1

have been reduced to approximately the level of the jars bubbled with CO_2. The effects of CO_2 are thus shown to have been directly due to this gas and not to the exclusion of O_2. Aeration generally increased the absorption of potassium and of phosphorus by maize, while covering maize culture solutions with paraffin oil to reduce O_2 absorption and CO_2 loss reduced the

TABLE II

THE ABSORPTION OF WATER AND MINERALS BY MAIZE PLANTS RECEIVING DIFFERENT TREATMENTS

SUBSTANCE ABSORBED	CONTROL	COVERED WITH OIL	BUBBLED WITH		COMPOSITION OF ORIGINAL SOLUTION
			AIR	CO_2	
EXPERIMENT III. FEBRUARY, 1943					
Water (ml.)	33.1 ± 2.7	32.7 ± 2.5	38.9 ± 4.2	28.4 ± 2.6	800.0
Total salts (mg.)	68.3 ± 4.2	64.2 ± 9.4	120.2 ± 7.0	42.2 ± 4.5	952.3
N	10.2 ± 2.6	5.5 ± 2.6	9.6 ± 2.9	3.7 ± 2.4	44.1
P	1.5 ± 0.3	1.0 ± 0.2	4.1 ± 0.2	0.7 ± 0.2	29.8
Ca	8.9 ± 1.7	8.9 ± 0.9	10.7 ± 0.3	5.1 ± 1.2	95.0
Mg	4.1 ± 0.6	4.1 ± 0.7	6.3 ± 0.6	2.7 ± 0.9	49.7
K	33.1 ± 1.1	17.5 ± 1.7	42.8 ± 2.5	*−7.8 ± 0.9*	138.4
EXPERIMENT IV. MARCH, 1943					
Water (ml.)	89.2 ± 5.3	88.5 ± 4.3	97.6 ± 4.4	71.2 ± 3.8	800.0
Total salts (mg.)	189.7 ± 7.6	174.0 ± 6.3	265.4 ± 6.7	97.8 ± 4.7	948.5
N	43.9 ± 1.6	41.3 ± 1.4	44.2 ± 1.6	16.1 ± 0.7	51.2
P	4.0 ± 0.4	4.6 ± 0.4	8.3 ± 0.4	0.9 ± 0.2	30.4
Ca	13.1 ± 1.2	15.6 ± 1.3	20.5 ± 1.6	9.7 ± 0.8	96.1
Mg	7.7 ± 0.4	6.7 ± 0.3	8.9 ± 0.5	5.4 ± 0.4	49.4
K	31.1 ± 1.6	15.8 ± 2.7	51.9 ± 2.0	0.5 ± 2.3	140.7

TABLE III

THE ABSORPTION OF WATER AND MINERALS BY RICE PLANTS RECEIVING DIFFERENT TREATMENTS*

SUBSTANCE ABSORBED	CONTROL	BUBBLED WITH		COMPOSITION OF ORIGINAL SOLUTION
		AIR	CO_2	
NO ADDED ACID, pH OF CONTROLS 5.0–6.0				
Water (ml.)	139.4 ± 7.4	155.5 ± 14.2	70.0 ± 14.6	800.0
Total salts (mg.)	48.8 ± 3.6	72.6 ± 4.5	1.6 ± 13.8	899.6
Nitrogen	8.0 ± 0.3	11.5 ± 1.1	0.9 ± 0.8	52.7
ADDED H_2SO_4, pH OF CONTROLS 4.0				
Water (ml.)	133.9 ± 12.9	133.1 ± 4.8	73.4 ± 12.6	800.0
Total salts (mg.)	16.4 ± 3.8	19.5 ± 3.6	7.3 ± 10.7	899.6
Nitrogen	5.1 ± 0.6	5.5 ± 0.2	0.6 ± 0.7	52.7

*Experiment V. August, 1943.

absorption of potassium. Other differences were either not statistically significant or were erratic. Adding sulphuric acid to bring the pH of rice cultures growing at high temperatures to 4.0 significantly reduced total salt and nitrogen absorption, but the effect of CO_2 was much greater than the effect of H_2SO_4.

To facilitate comparisons of the effects of the several treatments on the absorption of the different substances the data were calculated to show the treatments as a percentage of the controls and grouped by substances absorbed.

THE EFFECT OF CO_2 ON ABSORPTION OF WATER

The effects of aeration and CO_2 on the water relations of the plants were measured by weighing the culture solutions without the plants before and after the treatment period. The data are thus direct measurements of water absorption. Since, however, the plants were turgid at the beginning and at least nominally so at the end of the experiments, they are also measurements of transpiration. KRAMER's data (**15**) on the resistance of the root

TABLE IV

THE ABSORPTION OF WATER BY CONTROL AND TREATED PLANTS. CONTROL DATA IN MILLILITERS, OTHERS IN PERCENTAGE OF CONTROL

EXPERIMENT	CONTROL	AERATED	NITROGEN	OILED	CO_2
	ml.	%	%	%	%
I—wheat	62.4	112.2	.....	.....	65.1
II—wheat	53.5	106.0	112.9	.....	73.8
III—maize	33.1	117.5	.....	98.8	85.8
IV—maize	89.2	109.4	.....	99.2	79.8
Va—rice control	139.4	111.5	.....	.....	50.2
Vb—plus acid	133.9	99.4	.....	.....	54.8
Average (%)	100.0	109.3	112.9	99.0	68.3

system to water movement, and his later observations (**16**) that this friction was increased by CO_2, indicate that differences in absorption rather than transpiration were determining. This conclusion is supported by KRAMER's (**16**) experiments in which bleeding from decapitated plants under suction was reduced by the same or a greater percentage as was transpiration of intact plants. HOAGLAND and BROYER (**9**) also found that CO_2 reduced the yields of sap obtained from decapitated plants under suction.

Our results agree with those of KRAMER in showing that increased CO_2 concentration around the roots reduced water absorption while nitrogen gas had little effect. The data are summarized in table IV. They suggest that the oxygen concentrations obtained had no effect upon water absorption or loss under the conditions of these experiments. The gain of 9.3 per cent. in water loss with aeration is significant at the 5 per cent. level but is no greater than the gain from bubbling with nitrogen, and both could be assigned to a reduction in the average CO_2 content of the solutions. The oil treatment, which probably reduced oxygen absorption more than CO_2 loss, because of the differential solubilities of the gases in oil, had no effect on water loss. The reduced water loss from the jars receiving CO_2 was statistically significant in all but one test and highly significant as an average of all experiments.

The addition of sulphuric acid to bring the control solutions to the same pH as the CO_2 treated did not significantly reduce water absorption by rice. JACOBS (**11**), however, has shown that CO_2 may have a much greater effect on the internal pH of solutions or tissues protected by fatty membranes than other acids producing comparable external pH values. He assumed that CO_2 itself or undissociated H_2CO_3 is able to penetrate xylene, cottonseed oil, or protoplasmic membranes which are impermeable or only slowly permeable to non-fat-soluble acids. Once across the membrane the CO_2 dissociates to produce its equilibrium pH, which is near 4.0 in distilled water. Both JACOBS (**12**) and SEIFRIZ (**22**) have shown that CO_2 may increase the viscosity of protoplasm, and we may assume that the permeability of the root membranes to water is decreased by the changes set up.

The apparently greater sensitivity of rice to CO_2 than either wheat or maize is probably explainable on the basis of the higher greenhouse temperatures prevailing during the rice experiment, 30° to 35° C., in contrast to 20° to 30° C. for the other experiments. CANNON (**4**) has shown that CO_2 is more toxic at higher temperatures. A greater percentage decrease in water absorption might also have resulted from the very high transpiration rates during the rice experiment, even had the effect on root permeability been no greater.

THE EFFECT OF CO_2 ON ABSORPTION OF MINERALS

The treatments used and the analyses made varied somewhat among the five experimental runs. The data in table V are grouped around each analysis for convenient comparison. Absorption of the five elements tested was

affected in the order K > N > P > Ca > Mg. The absorption of all five was significantly reduced by CO_2, with the percentage reduction in the absorption of Ca and Mg paralleling that of water, the relative absorption of P and N being reduced to about half that of water and potassium being released from the roots in considerable quantities in two of four experi-

TABLE V

THE ABSORPTION OF NUTRIENT ELEMENTS BY CONTROL AND TREATED PLANTS. CONTROL DATA IN MILLIGRAMS PER CULTURE; OTHERS IN PERCENTAGE OF CONTROL

EXPERIMENT	CONTROL	AERATED	NITROGEN	OILED	CO_2
	mg.	%	%	%	%
ABSORPTION OF CALCIUM					
I—wheat	3.1	45.2	.....	.....	0.6
II—wheat	10.3	95.1	102.9	.....	79.6
III—maize	8.9	120.2	.....	100.0	57.3
IV—maize	13.1	156.4	.....	119.1	74.0
Average (%)	100.0	104.2	102.9	109.5	52.9
ABSORPTION OF MAGNESIUM					
II—wheat	7.0	105.7	104.3	.....	55.7
III—maize	4.1	153.6	.....	100.0	65.8
IV—maize	7.7	115.6	.....	87.0	70.1
Average (%)	100.0	125.0	104.3	93.5	63.9
ABSORPTION OF POTASSIUM					
I—wheat	7.4	256.7	.....	.....	*−29.7*
II—wheat	24.0	115.8	106.3	.....	33.7
III—maize	33.1	129.3	.....	52.9	*−23.6*
IV—maize	31.1	166.9	.....	50.8	1.6
Average (%)	100.0	167.2	106.3	51.8	*− 4.5*
ABSORPTION OF PHOSPHORUS					
II—wheat	2.5	104.0	84.0	.....	64.0
III—maize	1.5	278.3	.....	66.7	46.7
IV—maize	4.0	207.5	.....	115.0	22.5
Average (%)	100.0	196.6	84.0	90.8	44.4
ABSORPTION OF NITROGEN					
I—wheat	11.1	161.3	.....	.....	18.9
II—wheat	18.0	97.8	95.0	.....	83.9
III—maize	10.2	94.1	.....	53.9	36.3
IV—maize	43.9	100.7	.....	94.1	36.7
Va—rice	8.0	143.7	.....	.....	11.2
Vb—plus acid	5.1	107.8	.....	.....	11.8
Average (%)	100.0	117.6	95.0	74.0	33.1

ments. The differences shown fit with the general concept of Ca and Mg as elements whose accumulation is least dependent upon metabolic effects, and of potassium as the element whose accumulation is most dependent upon a delicate balance of protoplasmic forces and reactions. Calcium and magnesium could have been excluded by the same reduced permeability assumed to have acted upon water absorption. The potassium excretion is not so

easily explained except as we assume that the accumulation of this element is dependent upon protoplasmic structure and/or negative charge, both of which were probably modified by the treatment. HOAGLAND and BROYER (**9**) found that CO_2 reduced Br accumulation, and VLAMIS and DAVIS (**26**) found that both K and Br were excreted from excised roots held with very low oxygen tensions or bubbled with CO_2.

Of the other treatments, aeration significantly increased potassium absorption in maize and wheat and phosphorus absorption in maize. No other effects were significant. Under certain conditions such absorption increases as are shown for K and P in the aerated cultures could be expected to increase plant growth (**3**). The results of KNIGHT (**14**), however, and particularly those of ARNON (**1**) suggest that such increases are the results of an improperly balanced solution rather than poor aeration, and that equally good results could be obtained by changing the salts or their concentrations. Bubbling the solutions with cylinder nitrogen for ten minutes of each hour, to give an O_2 reduction comparable to that obtained with CO_2, did not affect any of the reactions. Covering the solutions with paraffin oil reduced potassium absorption by maize to 50 per cent. of the control. No other effect was statistically significant.

Discussion

The evidence for a directly toxic effect of CO_2 on plant as well as animal protoplasm seems to be conclusive. JACOBS (**10**) used some plant materials in his research. FOX (**6**) worked with Nitella. VLAMIS and DAVIS (**26**) report, "CO_2 was immediately lethal to all plants. . . . Other experiments with barley have indicated that a 20 to 30 per cent. partial pressure of CO_2 is toxic even if the entire residual pressure of 70 to 80 per cent. consists of oxygen." Although they do not stress the point, the data of HOAGLAND and BROYER (**9**) suggest that the roots of tomato plants were quickly killed by bubbling CO_2 through the solutions in which they were immersed. Other points are not yet established, however. We do not know the mode of action of CO_2 on protoplasm, the concentrations necessary for toxicity under varying conditions, or the practical importance of CO_2 toxicity in the field.

THE TOXIC ACTION OF CO_2

FOX (**5**) has reviewed the earlier work, most of it with animals including man, on the narcotic effects of CO_2, and has shown (**6**) that sea water saturated with the gas increases the viscosity of the protoplasm of *Nitella clavata*. Cells treated for one hour recovered with aeration but those left in CO_2 for two or three hours were killed. SEIFRIZ (**22**) observed almost identical reactions when *Physarum polycephalum* plasmodia were exposed to 50 per cent. CO_2 in oxygen. Anesthesia, as shown by the cessation of protoplasmic streaming, was complete within 60 seconds. Concentrations as low as 10 per cent. CO_2 gave symptoms of anesthesia. Carbon dioxide was the best narcotic for slime mold protoplasm of the substances tested, while ether was ineffective.

Distilled water saturated with CO_2 has a pH of about 4.0. ARNON, FRATZKE, and JOHNSON (**2**) and others have shown that hydrogen ion becomes directly and markedly toxic only at pH's toward 3.0 in the external medium. The rapid penetration of CO_2 through protoplasmic membranes, however, may bring about toxic internal changes in pH that are not reached with other acids. JACOBS (**11**) found that carbon dioxide dissolved in $NaHCO_3$ buffer at pH 6.9 was as toxic as in water at pH 3.9 and as quickly increased the internal acidity of immersed flower petals. Such changes in cellular acidity would tend first to decrease and then to increase the dispersion of the average protoplasmic colloid as the pH passed down through the isoelectric point or points. The effect of CO_2 is, however, to increase the viscosity. Such a change could be due to reduced hydration or to changes in molecular arrangement.

Two compounds of carbonic acid with protein have been reported; the formation of carbamic acid on the amino groups (**23**) and the formation of hydrogen-bond salts on the same groups (**20**). Carbamic acid compounds of protein derivatives are known only from their salts, especially of the earth metals. Amino acid carbamates form calcium salts by displacement of the hydrogens of both the amino and carbamic carboxyls. In proteins such calcium salts could presumably be formed between adjoining molecules, resulting in an increase in viscosity.

PAULI and STENZINGER (**20**) object to the carbamate theory of CO_2 toxicity. They found that various dialyzed animal proteins became positively charged upon treating with CO_2, and postulated the formation of weakly ionized, hydrogen-bond compounds. Both the carbamates, if produced, and the hydrogen-bond compounds would tend to form on the more basic amino groups above the alpha carbon, or possibly on cyclic nitrogen atoms. The residual bonds of the alpha amino nitrogen are too weak, especially after the formation of peptid linkages in protein synthesis, to expect them to form hydrogen-bond compounds. Compounding of these extra nitrogen atoms, on the other hand, would be little if at all affected by the polymerization of the amino acids into proteins. The dissociation of hydrogen-bond compounds into RH^+ and $OCOOH^-$ ions would increase the positive charge of the proteins, as observed by PAULI, and decrease their dispersion. The formation of such compounds might also affect the folding of the protein chains and the structure of the protoplasm while cross linkages between adjoining protein molecules reacting with the two hydrogens of the same carbonic acid molecule could rapidly increase the viscosity of the protoplasm, as observed by FOX (**6**) and SEIFRIZ (**22**).

A combination of rapid penetration of protoplasmic membranes, increases of cellular acidity, and the formation of specific, but weakly bonded, protein compounds seems to be the best available explanation of CO_2 toxicity. These reactions would fit the picture of a compound universally present in living cells becoming increasingly toxic above certain threshold concentrations. The formation of hydrogen-bond linkages on non-alpha amino nitro-

gen allows not only for reversibility and mass effects but for specific differences in CO_2 toxicity. Those plants whose protoplasm contained the fewest diamino and cyclic nitrogen groups would be most resistant to the action of CO_2.

The practical importance of CO_2 toxicity

Atmospheric oxygen is depended upon as an ultimate hydrogen acceptor in the respiration of higher plants. We must assume therefore that soil or culture aeration is beneficial at minimal oxygen levels because it replaces this element. At the same time possible toxic accumulations of CO_2 are swept away. The problem, therefore, is one of minimum oxygen levels, maximum CO_2 tolerance, and expected gas concentrations around the roots of plants growing in various environments.

Minimum oxygen levels have been estimated in various ways by many investigators. Stewart and Berry (**24**) found 20 per cent. oxygen in the gas above the liquids used to be maximum for accumulation of K and Br by storage tissue slices. At 3.8 per cent. oxygen, K accumulation was 70 per cent. and Br 66 per cent. of maximum. At 1.4 per cent. oxygen, K was lost to the solution. Vlamis and Davis (**26**) found that excised roots lost K and Br to solutions under partial oxygen pressures of less than 1 per cent., but showed nearly maximum accumulation with 2.7 per cent. O_2. Cannon (**4**) found that roots of most plant species grew with as little as 0.5 per cent. O_2, and that maximum growth was obtained with from 2 to 8 per cent. O_2, depending upon the species and conditions. Taylor (**25**) reports that germination of wheat was reduced significantly by O_2 pressures of 5 per cent. and was 50 per cent. of normal at 1 per cent. O_2. Rice germination was 90 per cent. of normal in purified N_2, with more than one-half the normal rate. We may conclude from the data available that plant roots will develop and function normally with 5 to 10 per cent. O_2 maintained in the soil gases and slowly with 1 to 2 per cent. O_2.

Data on the effects of CO_2 are meager. Cannon (**4**) found that growth of many roots was checked promptly by 25 per cent. but willow roots grew in 45 per cent. CO_2 with O_2 normal or higher. Seifriz (**22**) reports that 10 per cent. CO_2 was minimum for narcosis and 25 per cent. gave a rapid reaction. Twenty to 30 per cent. of CO_2, even with 70 to 80 per cent. O_2, was rapidly toxic to barley in experiments of Vlamis and Davis (**26**). Knight (**14**) found that maize was killed in soil sealed with paraffin until CO_2 accumulations reached 11.4 per cent.

These data indicate that CO_2 concentrations below 10 per cent. in the soil gases are probably not highly toxic, but that concentrations above 15 or 20 per cent. may be lethal to some plants. Analyses of soil gases at depths of 6 to 12 inches by Leather (**17**) and Russell and Appleyard (**21**) agree in showing typical CO_2 contents of 1.0 per cent. or less with O_2 concentrations of about 19 to 20 per cent. Leather states, "The diffusion of gases through soils at a depth of 12–15 inches is so efficient as to warrant the con-

clusion that cultivation of the surface soil is unnecessary for purposes of aeration." LEATHER, however, found CO_2 high around the roots of plants. RUSSELL and APPLEYARD recorded concentrations as high as 9.1 per cent. under rapidly developing *Festuca ovina*, and KNIGHT (**14**) observed 30.6 per cent. CO_2 in a garden soil sealed with paraffin. KIDD (**13**) found CO_2 concentrations at a depth of 12 inches as high as 20 per cent. when 3 inches of green grass was buried in an 18-inch pit. Control values were 1.0 per cent. Seeds of *Brassica alba* did not germinate over the decaying grass.

The available data indicate: (a) that plant roots vary in their sensitivity to CO_2; (b) that typical soils are low in CO_2 and high in O_2 so that a high resistance to CO_2 need not be a part of the normal picture of root development; and (c) that a high concentration of CO_2 in the soil air depends upon its rapid production by decay or root respiration as well as upon a low rate of gas exchange. The observation that CO_2 accumulation parallels O_2 depletion in upland soils is significant and indicates that slightly toxic to toxic CO_2 concentrations of 10 to 20 per cent. are probably more common than limiting O_2 concentrations of 1 to 2 per cent. Marsh soils, however, may be low in both O_2 and CO_2. Gases from flooded rice fields in India were nearly pure methane (**17**), and VLAMIS and DAVIS (**26**) were able to grow better rice bubbled with methane than with air. In spite of this resistance to low O_2 levels, rice was killed by CO_2 as quickly as tomato or barley.

Finally, CO_2 concentrations which reduce water and mineral absorption by one species in a plant community, even though not strongly toxic, may markedly affect competition. MCCOMB and LOOMIS (**19**) have suggested for example that upland forest species are less tolerant of CO_2 than are grasses and prairie species. Decomposition of the high subsoil organic matter of prairie soils and respiration by grass roots would thus create conditions unfavorable to the invasion of grasslands by forest, particularly on heavy, poorly aerated soils. Treatments such as erosion, grazing, or cultivation which reduce the soil organic matter and/or the development of grass roots increase forest invasion.

Summary

Absorption of water by the roots of wheat, maize, and rice growing in water cultures was reduced 14 to 50 per cent. by bubbling CO_2 through the solutions for ten minutes out of each hour. Bubbling air through cultures at the same intervals increased water absorption 9 per cent.; in one experiment, bubbling with nitrogen increased absorption by 13 per cent. Adding H_2SO_4 to bring the solutions to the pH reached with CO_2 had no effect upon water absorption. None of the plants was visibly injured by treatments lasting 36 hours.

Carbon dioxide treatments reduced the absorption of five nutrient elements in the order $K > N > P > Ca > Mg$. Calcium and magnesium absorption was reduced about as much as water absorption. Potassium was excreted from the roots of many of the plants treated with CO_2. Nitrogen and phosphorus absorption were intermediately affected.

It is suggested that the toxic effect of CO_2 on plant protoplasm is associated with its specific ability to change the internal pH of cells and to form hydrogen-bond compounds with proteins. Reported maximum carbon dioxide concentrations in field soils rise above toxic levels, and CO_2 toxicity should be included with oxygen deficiency as a factor affecting plant development in poorly aerated soils. Because of the importance of roots in raising soil CO_2, this gas may be a factor in root competition, particularly in the deeper layers of heavy soils.

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IRON IN THE LEAVES AND CHLOROPLASTS OF SOME PLANTS IN RELATION TO THEIR CHLOROPHYLL CONTENT

L. JACOBSON

(WITH ELEVEN FIGURES)

Introduction

That lime-induced chlorosis can be alleviated by the application of iron salts has been recognized for a hundred years. As early as 1845, GRIS (**8**) treated chlorotic plants with iron and obtained positive results; his experiments have been repeatedly confirmed. Opinions differ widely, however, as to the comparative amount and state of iron in chlorotic leaves. Various investigators have presented evidence indicating that more, the same, or less iron is present in chlorotic than in green leaves. Those who have reported the first two conditions have, in general, explained chlorosis as an immobilization or inactivation of iron which occurs mainly, or at least to a greater extent, in chlorotic leaves than in green ones. Of the more recent workers, WALLACE (**20**) and VIDAL (**19**) found no relation between total iron and chlorophyll content in lime-induced chlorosis, but GILE and CARRERO (**5**) and CHAPMAN (**2**) did. OLSEN (**13**) was unable to relate total iron to chlorophyll content in plants grown in iron-deficient culture solution whereas SCHOLZ was able to find such a relationship.

Both MOORE (**12**) and GRIESSMEYER (**7**) demonstrated the qualitative presence of iron in chloroplasts by employing staining reactions. LEIBICH (**10**) found up to 82 per cent. of the leaf iron in the chloroplasts of spinach. HILL and LEHMANN (**9**) found the isolated chloroplasts of Claytonia to contain four times as much iron as would be expected were the iron equally distributed throughout the leaf.

That the iron in the leaf is not all in the same chemical combination and does not all perform the same function seems indicated by the necessity of iron for non-green plants and the presence of iron containing enzymes in all plants. Hence it is probable that only a fraction of the leaf iron is closely related to chlorophyll formation. Although PARSCHE (**15**) found only a poor correlation between total iron and chlorophyll, he did find that the water soluble (filterable) iron was, as a rule, higher in green leaves. OSERKOWSKY (**14**), working with pear, apricot, and peach leaves, developed a method for determining a fraction of iron which was proportional to the chlorophyll content. His method consisted of extracting the dried leaf material with 1.0 N hydrochloric acid. He then plotted this acid-soluble iron against the chlorophyll content and obtained a straight line which, when extrapolated, intersected the iron axis at some point other than the origin. The difference beween the iron axis intercept and the acid-soluble iron for a given sample represented the amount of iron directly proportional to the chlorophyll. OSERKOWSKY called this "active iron" by which he

meant a specific iron fraction, or fractions, active for the formation of chlorophyll. The difference between the iron axis intercept and the origin he called the "acid-soluble inactive iron."

In the work reported here, this concept of active and inactive iron has been confirmed and extended. The total iron and chlorophyll content have been studied with particular reference to quantitative relationships. Since the chloroplasts are the chlorophyll containing bodies of the leaf, iron determinations were made on isolated morphologically intact chloroplasts.

Materials and methods

Field material

Leaves were collected from Hardy pear trees about 25 years old. These trees were growing in calcareous soil and showed varying degrees of lime-induced chlorosis. Four sets of green and chlorotic leaf samples were collected at intervals during the growing season. These samples were placed in jars and held at 2.5° C. until used within 48 hours after collection.

For the chlorophyll determination, two samples of ten or fifteen leaves each were selected at random, cut into small pieces, thoroughly mixed, and two grams carefully weighed out. This process was carried out as rapidly as possible to minimize drying.

For dry weight, ash, and iron determinations, leaves were weighed and washed in water to remove loose dirt. Then the surfaces of each leaf were carefully rubbed with the fingers in 0.3 N hydrochloric acid in order to remove encrusted dirt and spray residues. The acid was changed frequently and the washing conducted as rapidly as possible. There were no signs of injury. After the acid washing, the leaves were thoroughly rinsed in distilled water and dried in a ventilated oven at 55° C. for 48 hours. The dried leaves were weighed and then ground in porcelain ball mills with glass marbles.

Greenhouse material

Two species of plants, Turkish tobacco and Golden Bantam corn, were grown in solution in the greenhouse in 8-liter asphaltum painted stoneware crocks. The solution (half strength Hoagland) had the following composition: KNO_3, 0.0025 M; $Ca(NO_3)_2$, 0.0025 M; $MgSO_4$, 0.0010 M; KH_2PO_4, 0.0005 M. In order to make certain that the plants would not suffer from minor element deficiency, B, Mn, Zn, Cu, Mo, V, Cr, Ni, Co, W, and Ti were added in the amounts recommended by Arnon (1). Aeration of solutions was provided by compressed air entering through sintered glass aerators.

The plants were transferred to fresh nutrient solution every three weeks. The pH of the fresh solution was 5.5. At the end of the three-week period, the pH was about 7.5 for the corn cultures and about 6.0 for the tobacco cultures. At first, the plants were given small, but adequate, amounts of iron (1.0 ppm.) occasionally to maintain normal color and growth. Later, the amounts of iron and the frequency of its addition were adjusted so that the

desired degree of chlorosis was produced. By this means, plants ranging from very chlorotic to deep green were obtained.

In each experiment the plants, both corn and tobacco, were harvested when they were about two feet tall and had eight or ten pairs of mature leaves. Only the mature or nearly mature leaves from the upper half of the plant were used. The midribs of all leaves, and the basal and apical thirds of the corn leaves, were removed with a brass knife and discarded. From these prepared samples, numerous discs of leaf tissue were punched out with a brass cork borer for the chlorophyll determinations. Usually from 1.00 to 1.50 gm. were taken for analysis. For the other determinations, the remainder of the leaf sample, about 100 to 500 gm., was weighed, washed, dried, reweighed and then ground in the manner previously described for pear leaves.

All chlorophyll determinations were carried out on fresh material. The samples were thoroughly ground with sand, a pinch of calcium carbonate, and 80 per cent. acetone. The ground material was washed into a filter and washing with 80 per cent. acetone continued until a volume of 100 ml. had been reached. The transmission of this, diluted when necessary, was determined in a photoelectric colorimeter similar to that described by EVELYN (**4**). The filter used had a transmission band of 6000 to 7000 Å. Solutions of pure carotene and xanthophyll gave the same reading as the solvent blank, indicating that the filter transmitted no light in the absorption range of the carotenoids. The instrument was calibrated with known amounts of pure chlorophylls *a* and *b*, obtained through the courtesy of DR. G. MACKINNEY of this University. Because of the relatively small difference (7 per cent.) between the calibration curves of the two chlorophylls, the ratio could vary considerably without introducing a serious error. The chlorophyll content of a group of samples determined with the colorimeter and with a Bausch and Lomb spectrophotometer agreed to about $\pm$ 5 per cent.

Total iron was determined by a modification of the method of SAYWELL and CUNNINGHAM (**16**). From 50 to 200 mgm. of dried leaf tissue were digested with 1 ml. concentrated H_2SO_4 and a few drops of 30 per cent. H_2O_2. The digest was diluted and treated with 1 ml. of 10 per cent. hydroxylamine hydrochloride. The solution was now adjusted to pH 5 with NH_4OH, using a stirring rod and Congo red paper as an external indicator. One ml. of a 0.1 per cent. alcoholic solution of ortho-phenanthroline monohydrate was added and the volume adjusted to 10.0 ml. The color intensity was determined in the previously described colorimeter, using a filter with a transmission band of 4400 to 4800 Å.

To determine the acid soluble iron, 200 to 500 mgm. of dried leaf tissue were extracted with 10.0 ml. of 1.0 N HCl in 15-ml. centrifuge tubes. The extraction period was 6 hours for corn and tobacco, 24 hours for pear. During extraction, the samples were shaken from time to time. At the end of extraction, the tubes were centrifuged, aliquots of the supernatant liquid evaporated to dryness, and iron determined as described.

Ash weights were determined by igniting a 1.00-gm. sample of the dried leaf tissue in a muffle at 650° C.

Results

Dry matter and ash weights

The percentage dry weight of chlorotic leaves is frequently much lower than that of green leaves. Table I shows that this relationship was present in young leaves of field material, but was less evident in older leaves and in the material grown in the green house. In pear leaves there was an accumulation of dry matter as the growing season progressed. With approximately the same chlorophyll content (about 0.8 per cent. in the deep green leaves) the dry matter increased from 29.8 to 40.7 per cent. between April and September. At the same time, the difference in dry matter between the chlorotic and green leaves of a set tended to diminish, so that at the beginning of the season, there was a difference of about 25 per cent. between the most chlorotic and the greenest samples, whereas near the end of the season the difference was about 10 per cent.

In the corn and tobacco leaves there were no significant differences in percentage of dry matter except in the greenest sample of each set. Since these plants were first grown under normal conditions and then transferred to iron-free culture solutions, the chlorotic tissues were perhaps able to draw upon the lower green leaves for mobile nutrients. The larger percentage of dry matter in the deepest green leaves was probably due to higher photosynthesis.

There appeared to be no consistent difference in the ash content of the dried chlorotic and green samples of a set. However, the percentage ash was frequently less when the percentage dry weight was high. This was particularly true with pear leaves collected early in the season and, to some extent, with corn and tobacco leaves. Presumably the increased dry matter diluted the ash. The change in the ash content was probably an indirect result of chlorosis.

Iron in whole leaf

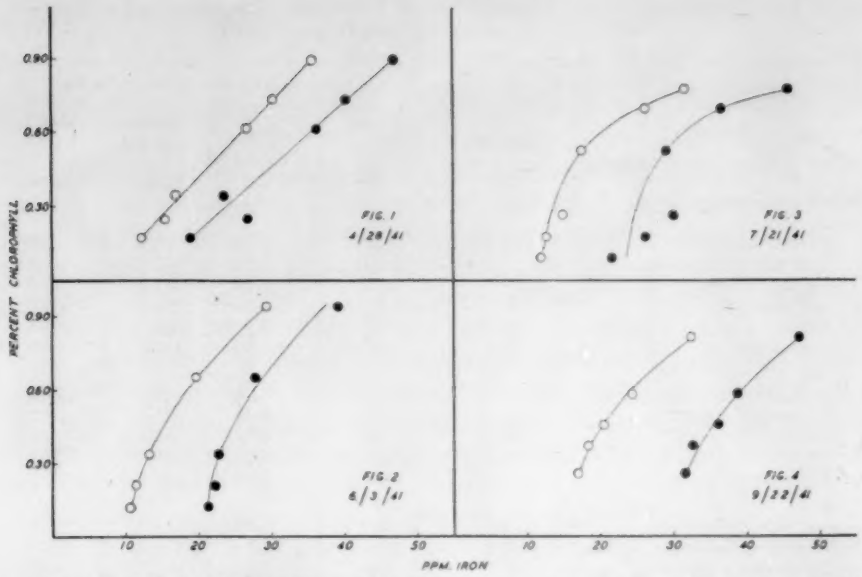
In table I, the data for the acid-soluble and total iron are given for several series of pear, corn, and tobacco leaves. These values are represented graphically in figures 1–8, inclusive. In every case the acid-soluble iron increased as the chlorophyll increased. As pointed out by Oserkowsky (**14**), it is only the iron which is proportional to the chlorophyll that can be considered as active in chlorophyll formation and not all of the acid-soluble fraction. The active iron fraction plotted against chlorophyll content displays a typical limiting factor type of curve. It is least evident in the pear leaf samples. In field material, one must select samples on the basis of greenness only, the range of iron content being determined by uncontrolled soil conditions. In greenhouse material, the iron content of samples is subject to some degree of control so that a large range of iron content in relation to the chlorophyll content could be obtained.

TABLE I

ANALYSES OF CHLOROTIC AND GREEN LEAVES

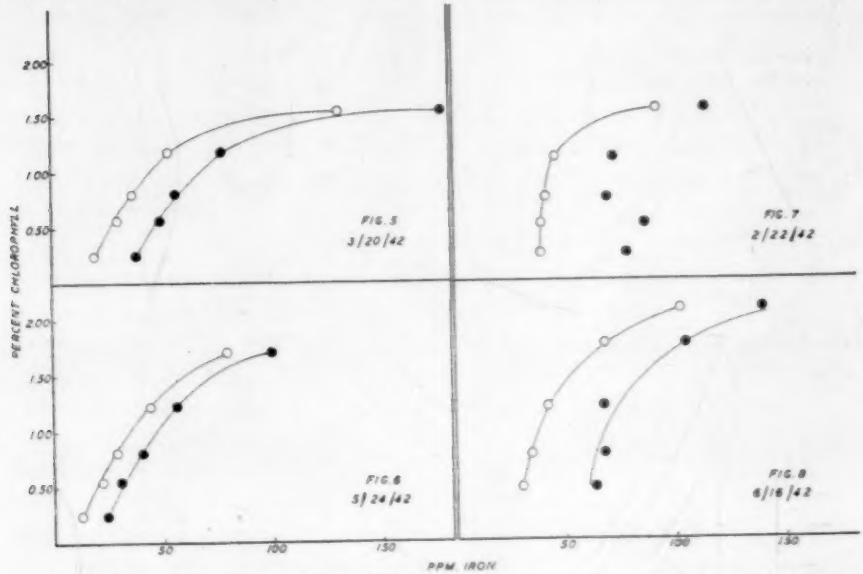
DESCRIPTION OF LEAVES	PERCENTAGE DRY WEIGHT	EXPRESSED ON DRY WEIGHT BASIS			
		CHLOROPHYLL	ASH	ACID-SOL. Fe	TOTAL Fe
	%	%	%	*ppm.*	*ppm.*
Hardy pear spur leaves, 4/28/41					
Severely chlorotic	22.3	0.170	6.78	12.4	19.0
Moderately chlorotic	23.9	0.248	6.14	15.6	26.9
Slightly chlorotic	25.0	0.341	6.02	17.0	23.6
Pale green	26.7	0.614	5.60	26.7	36.4
Green	28.3	0.735	4.90	30.4	40.3
Deep green	29.8	0.893	4.92	35.7	46.9
6/3/41					
Severely chlorotic	30.3	0.129	6.62	10.7	21.4
Moderately chlorotic	34.5	0.214	5.44	11.5	22.3
Slightly chlorotic	35.7	0.340	6.08	13.3	22.7
Green	36.7	0.654	6.16	19.8	27.8
Deep green	36.6	0.943	6.64	29.4	39.2
7/21/41					
Very sev. chlorotic	30.4	0.0883	8.50	12.0	21.8
Severely chlorotic	33.8	0.173	7.86	12.7	26.4
Moderately chlorotic	37.0	0.263	8.08	15.0	30.1
Pale green	39.6	0.525	8.08	17.6	29.2
Green	39.0	0.693	7.21	26.2	36.6
Deep green	37.3	0.777	7.46	31.7	45.8
9/22/41					
Moderately chlorotic	38.3	0.258	6.68	17.0	31.6
Slightly chlorotic	42.0	0.372	7.28	18.4	32.6
Pale green	42.4	0.460	7.32	20.4	36.1
Green	42.8	0.582	7.44	24.4	38.7
Deep green	40.7	0.816	7.64	32.8	47.2
Corn, 3/20/42					
Severely chlorotic	17.6	0.243	8.05	19.6	38.3
Moderately chlorotic	18.2	0.566	7.25	30.1	49.6
Pale green	17.5	0.798	7.15	36.9	56.5
Green	17.8	1.180	7.10	53.4	77.3
Deep green	19.3	1.540	7.20	131.0	178.0
Corn, 5/2/42					
Severely chlorotic	14.0	0.246	8.20	12.7	24.2
Moderately chlorotic	14.1	0.543	7.18	21.8	30.6
Pale green	14.9	0.810	7.35	28.8	40.8
Green	14.4	1.220	7.50	44.3	56.3
Deep green	16.1	1.710	6.83	79.4	100.0
Tobacco, 2/22/42					
Severely chlorotic	7.66	0.242	17.1	39.6	78.9
Moderately chlorotic	7.22	0.507	18.8	40.0	87.0
Pale green	7.82	0.743	17.3	42.1	70.2
Green	7.38	1.110	16.2	46.5	73.2
Deep green	8.67	1.540	14.9	92.7	115.0
Tobacco, 6/16/42					
Moderately chlorotic	8.89	0.471	14.5	30.1	63.7
Pale green	8.92	0.769	15.3	34.7	68.2
Green	9.41	1.200	13.6	42.3	67.7
Deep green	9.23	1.760	13.7	68.4	105.0
Very deep green	11.60	2.070	10.2	103.0	140.0

In all but one set, the total iron curve paralleled the acid-soluble curve. Since the total iron was found to be related to the chlorophyll content and this finding disagreed with much of the published data, this aspect was inves-



FIGS. 1, 2, 3, 4. Acid-soluble and total iron in pear leaves, expressed on a dry weight basis. ○, acid-soluble iron; ●, total iron.

tigated further. It is possible that the large number of leaves collected per sample, and the fact that selection was distributed among several trees or plants, may have tended to cut down the individual variation and thus give



FIGS. 5, 6. Corn leaves. Acid-soluble and total iron, expressed on dry weight basis. ○, acid-soluble; ●, total iron.

FIGS. 7, 8. Tobacco leaves. Acid-soluble and total iron, expressed on dry weight basis. ○, acid-soluble; ●, total iron.

more consistent results. Also the thorough washing employed could account for more consistent results. Very few investigators mention washing their samples preparatory to analysis.

CZAPEK (3) states that the usual iron oxide content of leaf ash is 1 to 4 per cent., sometimes lower and frequently higher. He then cites values as high as 10.2 per cent. (*Vitis vinifera*), 16.17 per cent. (*Triticum repens*) and even higher in other plants. The deep green sample of corn leaves harvested May 20, 1942 was the richest in iron of any sample analyzed. Yet its iron content, expressed as percentage iron oxide of ash, amounted to only 0.35 per cent. Even allowing for differences in species, the values reported by CZAPEK seem rather high in comparison to the above value. In view of this, it was felt that an examination of the magnitude of surface contamination was warranted.

Samples of chlorotic and green pear leaves were collected fom trees growing in calcareous soil and also, for comparison, a sample was collected

TABLE II

EFFECT OF WASHING ON LEAF IRON

TREATMENT OF LEAVES	PPM. FE OF DRY WEIGHT		
	CHLOROTIC LEAVES FROM CALCAREOUS SOIL	GREEN LEAVES FROM CALCAREOUS SOIL	GREEN LEAVES FROM NON-CALCAREOUS SOIL
	ppm.	*ppm.*	*ppm.*
Unwashed	348.0	250.0	547.0
Washed in water only	82.0	82.4	177.0
Washed in acid once	21.6	36.1	80.4
Washed in acid twice	20.0	36.1	79.5

from an orchard in non-calcareous soil. The leaves, which were quite dusty and covered with spray residue, were first washed carefully in distilled water, then in 0.3 N hydrochloric acid, rinsed thoroughly in distilled water, washed a second time in acid, rinsed again, and dried. The iron was determined in the dried leaves and various wash waters. Since the volumes of the latter were known, as well as the dry weights of the leaves, the respective amounts of iron in unwashed leaves, water washed, and acid washed leaves were calculated. The data are presented in table II.

It is evident that analyzing field samples for iron without preliminary washing may cause a 1700 per cent. error (compare acid-washed and unwashed chlorotic sample). This may be the explanation for the extremely high iron values cited by some authors. It is further to be noted that washing in water alone is not sufficient: the leaves still contain enough surface contamination to cause more than a 100 per cent. error. The first acid washing removed practically all remaining surface iron, since the second removed no more than traces. The method of acid washing as used here probably does not leach out any iron from the interior of the leaf. Such

leaching would require that the acid penetrate into the leaf but since no signs of injury were observed, it is doubtful if this occurred. All of the above considerations would apply to greenhouse-grown material, although to a lesser extent, since these plants were not exposed so long or to so much wind-blown dust as orchard trees. Moreover, the orchard trees were sprayed while the greenhouse plants were not. Much of the surface iron on leaves from the former may have been in the spray materials.

Chloroplast iron

Chloroplasts were isolated from three sets of tobacco plants by the procedure of Granick (**6**), except that the leaves were ground in a "blendor" instead of with mortar and pestle. Essentially, the method consisted of

TABLE III

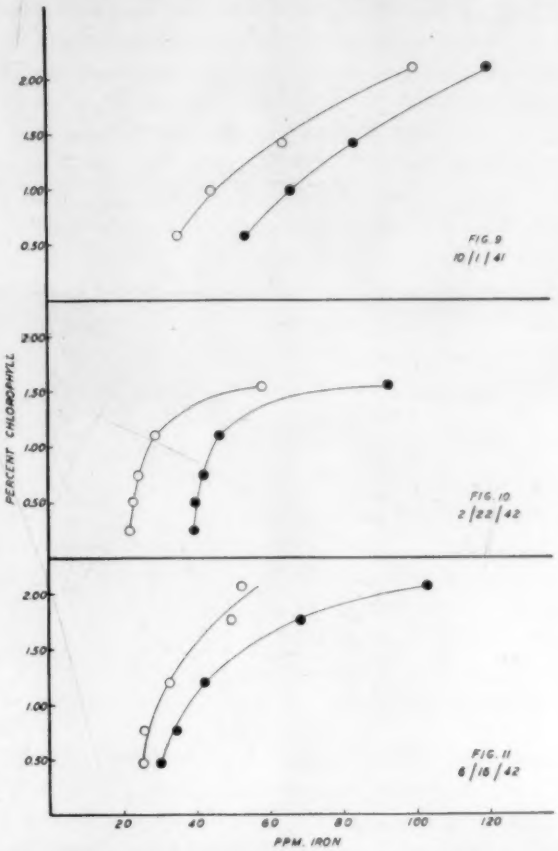
Acid-soluble iron in whole leaf tissue and chloroplasts of tobacco, calculated on dry weight basis

Date	Percentage chlorophyll of whole leaf	Ppm. acid-soluble iron expressed on whole leaf basis	
		Whole leaf	Chloroplasts
	%	*ppm.*	*ppm.*
10/1/41	0.584	53.8	35.3
	0.989	66.3	44.8
	1.430	83.5	64.3
	2.110	120.0	100.0
2/22/42	0.242	39.6	22.0
	0.507	40.0	23.0
	0.743	42.1	24.5
	1.110	46.5	29.0
	1.540	92.7	58.1
6/16/42	0.471	30.1	25.3
	0.769	34.7	25.7
	1.200	42.3	32.7
	1.760	68.4	49.4
	2.070	103.0	52.6

liberating the chloroplasts in 0.5 molar glucose solution and isolating them by differential centrifugation at controlled centrifugal forces. Microscopic examination showed the presence of only chloroplasts and starch granules in the preparations. The plastids as well as the original leaf material were analyzed for acid-soluble iron and chlorophyll. From these values, the fraction of acid-soluble leaf iron, localized in the chloroplasts, was calculated. In table III, the chlorophyll content and acid-soluble iron of whole leaf tissue, as well as chloroplast acid-soluble iron, are given. Figures 9, 10, and 11 are graphical representations of this data. In the region where iron is limiting, the acid-soluble iron of the chloroplasts parallels that of the whole leaf; i.e., active iron can be determined from either curve. Since the acid-soluble iron in the non-chloroplast portions of the leaf remains essentially constant when iron is a limiting factor, the active iron (fraction of iron directly proportional to chlorophyll content) must be localized in the chloro-

plasts. As pointed out by OSERKOWSKY, there is no stoichiometric relation between this fraction of iron and chlorophyll content. This is shown graphically by the different slopes for each set of data.

Because of the structure of corn leaves, a comparison between the isolated chloroplasts and whole leaf tissue was not possible. In chlorotic corn leaves, the interveinal region is much lighter in color than the region immediately adjacent to the vascular bundle. Because of the fibrous nature of the latter,



FIGS. 9, 10, 11. Acid-soluble iron in chloroplasts and whole leaf tissue of tobacco, expressed on dry weight basis of whole leaf. ○, chloroplasts; ●, whole leaf tissue.

these greener cells are not readily ruptured by the grinding process and therefore one does not obtain representative chloroplast samples. This was not the case with tobacco leaves, in which grinding ruptures more or less the same proportion of cells regardless of position in the leaf.

However, microscopically clean chloroplast preparations (though not representative) could be obtained from corn leaves and analyses made of them. In table IV, acid-soluble and total chloroplast iron are presented in terms of micrograms per ml. of chloroplast suspension for both corn and

tobacco. From 57 to 74 per cent. of the total iron in the chloroplast is acid-soluble. There are no particular trends: in two cases, the chlorotic samples have a greater percentage of acid-soluble iron than the green samples; in the others, the converse is true.

In the tobacco samples, for which the active iron values were determined, there is a decided difference between the chlorotic and green plastids with respect to this fraction. The chlorotic chloroplasts contain a much smaller percentage of iron in the active form than do the green plastids. On the other hand, the change in slope of the chloroplast curves in figures 9, 10,

TABLE IV

IRON FRACTIONS IN CHLOROPLASTS

DATE	DESCRIPTION	MICROGRAMS PER ML. OF CHLOROPLAST SUSPENSION		
		ACTIVE Fe	ACID-SOL. Fe	TOTAL Fe
		γ	γ	γ
	Corn			
3/20/42	Chlorotic	.....	1.210 (65.1%)*	1.860
	Green	.....	3.310 (57.9%)	5.720
5/2/42	Chlorotic	.....	0.519 (67.9%)	0.764
	Green	.....	3.260 (65.2%)	5.000
	Tobacco			
2/22/42	Chlorotic	0.0567 (3.73%)	0.931 (61.3%)	1.520
	Green	1.120 (29.3%)	2.830 (74.1%)	3.820
6/16/42	Chlorotic	0.138 (9.08%)	0.865 (56.9%)	1.520
	Green	0.884 (36.5%)	1.570 (64.9%)	2.420

* Indicates percentage of total iron.

and 11 indicate that at low chlorophyll values, whatever active iron is present is utilized more efficiently than at high chlorophyll values.

In recent years, the idea that chlorophyll exists in the leaf in combination with a protein has received considerable attention. Since MOMMAERTS (11) reported the presence of iron in a purified chlorophyll-protein complex, such a complex was isolated from both corn and tobacco leaves and investigated. The complex was prepared by the ammonium sulphate method of SMITH (18). Only carefully tested iron-free reagents were used. Iron was found to be present in significant amounts in all preparations.

Since slightly alkaline solutions had been used, the possibility existed that iron had been precipitated by the alkali, so the procedure was carried out using ammonium sulphate adjusted to pH 5.0. Although the protein complex appeared to have become denatured as a result of the acidity, iron was still present. This suggests that the iron was closely associated with a

protein. Experiments with papain, to be described in a later paper, tend to confirm this supposition.

Discussion

If the acid-soluble iron be plotted against the varying chlorophyll content for a series of samples grown under similar conditions, a curve is obtained which does not pass through the origin, but intersects the iron axis. Two assumptions are made: (1) that the iron given by this intercept is constant throughout the set; and (2) that it is inactive in chlorophyll formation. These two assumptions provide the simplest interpretation of, and are consistent with, the observed data. Even if the inactive iron were not constant but varied uniformly, either decreasing or increasing with chlorophyll content, the general concept would not be changed. The quantitative relationships, however, would be altered.

In all but one set of samples, the curve for total iron parallels that for acid-soluble iron. That is, the active iron values are the same whether derived from either the acid-soluble or total iron curve. This would indicate that the chlorophyll-forming mechanism involving iron is the first to suffer when iron becomes deficient. The intercept of the total iron curve with the iron axis denotes the minimum amount of iron necessary for the maintenance of the leaf aside from chlorophyll formation. When iron is a limiting factor, there is assumed to be no partition of it among the various fractions when it enters the cell; but rather it is suggested that certain other requirements (iron containing enzymes, etc.) must be satisfied before active iron and chlorophyll can be formed.

The slope of the active iron curve is not constant; it is greatest in the chlorotic region. Although chlorotic leaves are low in active iron, whatever active iron is present is used more efficiently than in green leaves. The variation of the active iron efficiency (slope) between different sets shows that other factors, internal or external, determine the quantitative relation between active iron and chlorophyll.

Because chlorosis may be cured by treatment with iron salts and yet analyses apparently show chlorotic leaves to contain as much or more iron than green ones, it has frequently been assumed that abnormal inactivation or precipitation of iron occurs in chlorotic leaves. However, washing experiments indicated that this entire problem required examination. It was found that with few exceptions properly acid-washed chlorotic leaves contained less total iron than green leaves.

The probable source of the surface contamination is spray materials and dust particles which contain iron as the oxide or silicate. Both of these are essentially insoluble in dilute acid and hence, even if present, would not appear in the acid-soluble fraction. Confirmation of this was obtained by the fact that the iron in the acid wash water could be removed by centrifugation and only reacted with iron reagents after boiling with strong acid for several minutes. The efficacy of the acid washing does not depend on the dissolv-

ing off of the iron contamination, but rather on dissolving the spray materials and permitting the dust particles to be more readily dispersed in the wash solution.

An analysis of isolated chloroplasts clearly indicates that the active iron fraction is localized in the chloroplasts, although the latter also contain acid-soluble inactive iron. The nonchloroplast, acid-soluble iron of the cell remains approximately constant as long as iron is a limiting factor. When iron is no longer limiting in chlorophyll formation, it accumulates more rapidly in the nonchloroplast fraction of the cell than in the chloroplasts. The presence of acid-soluble iron throughout the cell is in agreement with the work of MOORE (**12**) who found a labile (reacting with hematoxylin) fraction of iron in the chloroplasts, cytoplasm, nucleus, and occasionally in the cell wall.

Summary

1. The active iron concept of OSERKOWSKY is valid for corn and tobacco leaves as well as for pear leaves.

2. A proportionality exists between total iron and chlorophyll content in the leaves of the plants studied.

3. Before chlorophyll formation can occur, the total iron content of the leaf must exceed a certain minimum level, which is determined by the species and the growth conditions.

4. Thorough washing of the leaves with dilute acid is a prerequisite for valid quantitative analyses of iron. Neglect of this point may lead to highly erroneous results.

5. In tobacco, the active iron is localized solely in the chloroplasts; but other fractions, both acid-soluble and acid-insoluble, are also present.

6. Iron is present in the chlorophyll-protein complex prepared by ammonium sulphate precipitation from corn and tobacco leaves.

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THE EFFECT OF REDUCED LIGHT INTENSITY ON THE AERIAL AND SUBTERRANEAN PARTS OF THE EUROPEAN BINDWEED[1]

A. L. Bakke and W. G. Gaessler

(with two figures)

Introduction

In excavations 4 × 4 feet made at different times between 1933 and 1937 at Hawarden and Cherokee, Iowa, to determine the extent of the root system of the European bindweed, *Convolvulus arvensis* L., no outgrowth of aerial shoots was observed below a depth of approximately 12 feet. The light intensity at the place where no aerial growth took place, as measured by a Weston light meter at 2:00 P.M. on September 19, 1936, was 42 f.c. while the reading in direct sunlight at that time was 7,500 f.c. There was a gradual increase in the number of aerial shoots above the 12-foot point which suggested that insufficient light was the principal factor limiting the development of green shoots at the lower depths. The object of the investigation reported in this paper was to determine the effect of reduced light upon the aerial and subterranean parts of the European bindweed and to gain some specific information concerning the depletion of root reserves, resulting from a prolonged period of light reduction. Along with this objective an effort was made to determine whether the principle of light reduction may be effectively used in the eradication of perennial weeds like the European bindweed.

The contributions to the rôle of light made by MacDougal (**18**), Lubimenko (**16**), Thatcher (**26**), Combes (**10**), Rosé (**21**), Shantz (**23**), Garner and Allard (**12**), Gourley (**13**), Gourley and Nightingale (**14**), Schultz and Thompson (**22**), Lubimenko and Karisnev (**17**), Bates and Roeser (**7**), Iwanoff and Kossowitsch (**15**), Weaver and Clements (**28**), Tyson (**27**), Clements and Long (**8, 9**), and Shirley (**24, 25**) have shown that a reduction in light intensity produces plants with attenuated stems, and thin walled, loosely organized leaf cells; reduces root development and root reserves; decreases the percentage of dry matter and stored carbohydrates; increases the moisture content, reducing sugars, sucrose, water soluble nitrogen, and ash content; and prolongs vegetative and fruiting periods. Bakke (**3**) disclosed that shaded European bindweed plants lost their normal prostrate form and became twining plants, but the literature presents very little information on the effect of reduced light upon the food reserves in the roots of perennial plants.

[1] Contribution from the Iowa Agricultural Experiment Station in cooperation with the Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, Iowa State Board of Control (Cherokee State Hospital, Cherokee, Iowa).

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Materials and methods

Four cages 4 feet wide, 4 feet high, and 6 feet long were made of 2- × 2-inch fir and covered with hardware cloth except at the bottom and in turn these cages were overspread with single or multiple layers of cloth of various kinds so as to transmit various amounts of light. The top of each cage was provided with a hinged door so that light, evaporation, and temperature readings could be readily made. The four cages were numbered and covered as follows: (1) two layers of aster cloth; (2) one layer white muslin; (3) one layer of light canvas or drill; (4) two layers of black cloth (cambric). The cages were set up on July 26, 1937, on the grounds of the Cherokee State Hospital, Cherokee, Iowa, directly over an area heavily and uniformly infested with European bindweed. A control plot of the same area (24 square feet) was included. The location of the respective plots was at random and the plots placed at a distance of a rod from each other in a direct south and north line. The tops of the bindweeds were cut off at the crown from the entire area at the time the experiment was begun. The cages were then placed in the following order going from south to north: (1) two layers aster cloth; (3) one layer light canvas (drill); (2) one layer of white muslin; (5) control; (4) two layers black cloth (cambric). The bindweeds immediately surrounding the chambers were removed and kept from developing by weekly hoeings each year. The area to the east and west for about two rods was sprayed in 1937 with sodium chlorate. The cages were kept in place throughout the entire growing season, being reset each spring. The study was continued for four years (1937–1940) or until there was practically no aerial growth in the drill chamber (No. 3). None of the plots was cultivated during that time. The edges of the cages were carefully protected from light by being partly covered with nearby soil. Usually new cloth replaced that of the year before.

Leaf areas were determined by the aid of standard cross-section paper, and the thickness of the leaves was measured by a micrometer caliper. During the first season daily light measurements were made with a Weston light meter. During the 1937 season, measurements of light intensity and temperature were made at least once a day at a time when light was most intense and from July 28 to August 22 twice a day, in the morning and again in the afternoon. The evaporating environment in three of the habitats (No. 1, two layers aster cloth; No. 3, drill; and No. 5, control) was determined by ascertaining the evaporation from standardized black and white spherical LIVINGSTON atmometers. Thickness and area of leaves of the bindweeds growing in the various areas were measured in 1937. At the end of each growing season, shortly before time of killing frost, the amount of aerial bindweed growth was determined by weighing.

At the end of the fourth season the roots at depths of one, two, and three feet were collected separately and weighed. Determinations of moisture content, nitrogen, total reducing sugars, starch, and total available carbohydrates were made as previously described by BAKKE *et al.* (**5**, **6**).

Experimental results

The data obtained of the daily measurements of light intensity, temperature and evaporating environment from July 26 through August and part of September, 1937, have been grouped and placed as averages for the respective months. As shelter No. 4 showed a light intensity less than one foot candle, no readings were taken. The data are set forth in table I.

The light intensity and temperatures in the four habitats, according to results from July 28 to August 22, generally showed a higher intensity in the afternoon than in the morning. The light intensity inside the two layers of aster cloth ranged from 200 to 3,400 f.c.; inside the one layer of muslin 84 to 1,150 f.c.; inside the one layer of drill 51 to 650 f.c.; and in the open 1,400 to 10,000 f.c. The mean temperatures of the different habitats varied

TABLE I

READINGS OF LIGHT INTENSITY, TEMPERATURE, AND EVAPORATION IN HABITATS EXPOSED TO VARIOUS AMOUNTS OF LIGHT, 1937

TIME	AVERAGE LIGHT INTENSITY IN CAGES*				MEAN TEMP.†		AVERAGE RADIANT ENERGY 24-HR. PERIODS‡		
	1	2	3	5	MAXIMUM	MINIMUM	1	2	5
	f.c.	*f.c.*	*f.c.*	*f.c.*	°*F.*	°*F.*	*cc.*	*cc.*	*cc.*
July 26–31	2064	820	490	7067	87.1	58.5	6.27	1.46	12.42
August	2234	549	305	8181	88.6	66.5	4.24	1.02	13.41
September	1397	168	92	7403	77.2	52.5	2.74	0.82	10.87

* Description of shelters: 1, two layers aster cloth; 2, one layer muslin; 3, one layer drill; 4, two layers black cloth—not included; 5, no shading—open.

† Cherokee, Iowa, Weather Bureau Station.

‡ Average differences between standardized Livingston white and black spherical atmometers (radiant energy).

from 62° to 108° F. Evaporation as measured by the black spherical atmometers ranged from 1.67 to 33.06 cc. per 24 hrs. in the No. 1 environment; 1.50 to 19.50 cc. per 24 hrs. in the No. 3 habitat; and in No. 5 (sunlight) 15.5 to 92.11 cc. per 24 hrs. The evaporation, as determined by the white spherical atmometers for one day, ranged as follows: No. 1, 0.48 to 28.64 cc.; No. 3, 0.40 to 18.08 cc.; No. 5, 11.04 to 79.19 cc. The differences between the white and black spherical atmometers measuring the radiant energy were as follows: No. 1, 0.40 to 16.22 cc.; No. 2, 0.04 to 3.97 cc.; No. 5, 4.46 to 17.28 cc. On the basis of these data it is clear that at times the evaporating environment was high.

Daily measurements of light, temperature, and evaporation were not made after the 1937 season. Light intensities were measured from time to time during the growing seasons of 1938 to 1940, and were in line with the results of 1937. According to REED (**20**) the summer season June, July, August, 1938 averaged warmer and drier than normal. It was the eighth consecutive dry summer. The summer season of 1939 averaged warmer

TABLE II

MONTHLY EVAPORATION (INCHES) FOR THE SUMMER MONTHS AS MEASURED BY THE CHEROKEE, IOWA, STATION OF THE UNITED STATES WEATHER BUREAU

	YEAR			
	1937	1938	1939	1940
June	.....	7.224	7.200	8.880
July	9.187 (Ames)	9.083	9.239	9.389
August	7.746	8.165	6.840	5.392
September	6.425	5.133	8.762	5.744

and wetter than normal. The evaporation during September was unusually high, as is noted from table II. The 1940 season was wetter and slightly warmer than normal.

The thickness and area of leaves of European bindweed grown under different light intensities were determined in August and September 1937. Averages of these determinations are given in table III.

European bindweed plants grown in a light intensity of 650 f.c. (cage No. 3) had a leaf thickness about one-half that recorded for the leaves of similar plants grown in the open. The average leaf size of plants in shelter No. 3 (one layer drill) was approximately the same as the average for leaves developed under full sunlight.

To determine the effect of decreased light intensity upon the amount of leaf and stem tissues formed, the green tissue was weighed at the end of each season from 1937 to 1940 (fig. 1). The data are given in table IV. On May 21, 1940, a time when the shoots of the European bindweed in undisturbed areas had taken on an active growth, there were no plants in cage 4 (black cloth), none in No. 3 (drill), 6 plants in cage No. 2 (muslin), 10 in cage 1, and 65 in the control.

Assuming that the amount of leaf and stem tissue was approximately the same in all cages at the beginning of the experiment July 26, 1937, and would continue to so remain if the environment had remained the same, the reduction in the amount of above-ground material the first season was small, perhaps not significant. In case of shelter No. 2 (one-layer muslin) the weight of

TABLE III

AVERAGE THICKNESS AND AREA OF EUROPEAN BINDWEED LEAVES IN VARIOUS LIGHT INTENSITIES

CAGE NO.	CAGE COVERED WITH	MAXIMUM LIGHT INTENSITY	AVERAGE LEAF	
			THICKNESS	AREA
		f.c.	*mm.*	*sq. cm.*
1	Two layers aster cloth	3,400	0.220	8.15
2	One layer muslin	1,150	0.210	7.15
3	One layer drill	650	0.190	3.76
4	Two layers black cambric	< 1	0.160	0.69
5	Open	10,000	0.334	3.21

the leaves and stems was 255.2 grams higher than the control. At the end of the 1938 season, the total green weight present in each of the 5 habitats was considerably less than that of the previous year. Here shelter No. 2 had only 24.8 grams of green tissue, somewhat lower than No. 4 where the light intensity was less than one foot candle. It was mere coincidence that the green weight of No. 1 and of No. 4 was the same. At the end of the 1939 season there was a marked reduction in leaf and stem tissue for shelters Nos. 1, 3, and 4, but an increase in the one-layer muslin shelter. At the end of 1940 there was a general decrease in the amount of green tissue with the exception of No. 4 where the light intensity was less than one foot candle. This apparent discrepancy can be accounted for by the fact that the No. 4 cage was placed on the north edge of the infested area and became infested after 1939

TABLE IV

AMOUNT OF GREEN AERIAL EUROPEAN BINDWEED TISSUE AT THE END OF RESPECTIVE GROWING SEASONS, 1937–1940, WHEN AN ORIGINALLY HEAVY INFESTED AREA WAS SUBJECTED TO DIFFERENT LIGHT INTENSITIES

DESIGNATION OF SHELTERS	GRAMS OF GREEN MATERIAL (LEAVES AND STEMS) PER 24 SQ. FT. AT END OF SEASON			
	1937	1938	1939	1940
	gm.	*gm.*	*gm.*	*gm.*
No. 1—Two layers aster cloth	1389.1	893.8	170.1	4.5
No. 2—One layer muslin	1956.1	24.8	113.4	3.6
No. 3—One layer drill	1418.5	60.2	2.1	0.7
No. 4—Two layers black cloth	992.2	35.5	3.0	65.5
No. 5—Open	1700.9	893.8	1460.8	276.0

through the underground growth of the roots of the unmolested bindweeds of adjoining wheat field. In 1940 the amount of green weight in shelters No. 2 and 3 was small; in 1941 there was no aerial growth.

The reduction in the amount of aerial growth in the control from 1,700.9 in 1937 to 276 grams in 1940 no doubt was due to the fact that the roots became matted and caused a local competition since the area was not cultivated.

Sufficient evidence is presented to show that continued reduction in the amount of light reduces normal photosynthesis and in turn produces a reduction in the amount of aerial tissue, other conditions being equal. There is evidence, too, that where light is reduced the most, the amount of aerial tissue produced is least. In other words, the lower the light intensity, the more rapid will be the elimination of the above-ground parts.

To determine the effect of four years of shading on bindweed roots, excavations of root material were made in the first three feet in the various areas on October 31, 1940. The moisture content, green weight, dry weight, amount of available carbohydrates and percentages of nitrogen, total reducing sugars, starch, and total available carbohydrates (fig. 2) were determined. The results have been tabulated in table V.

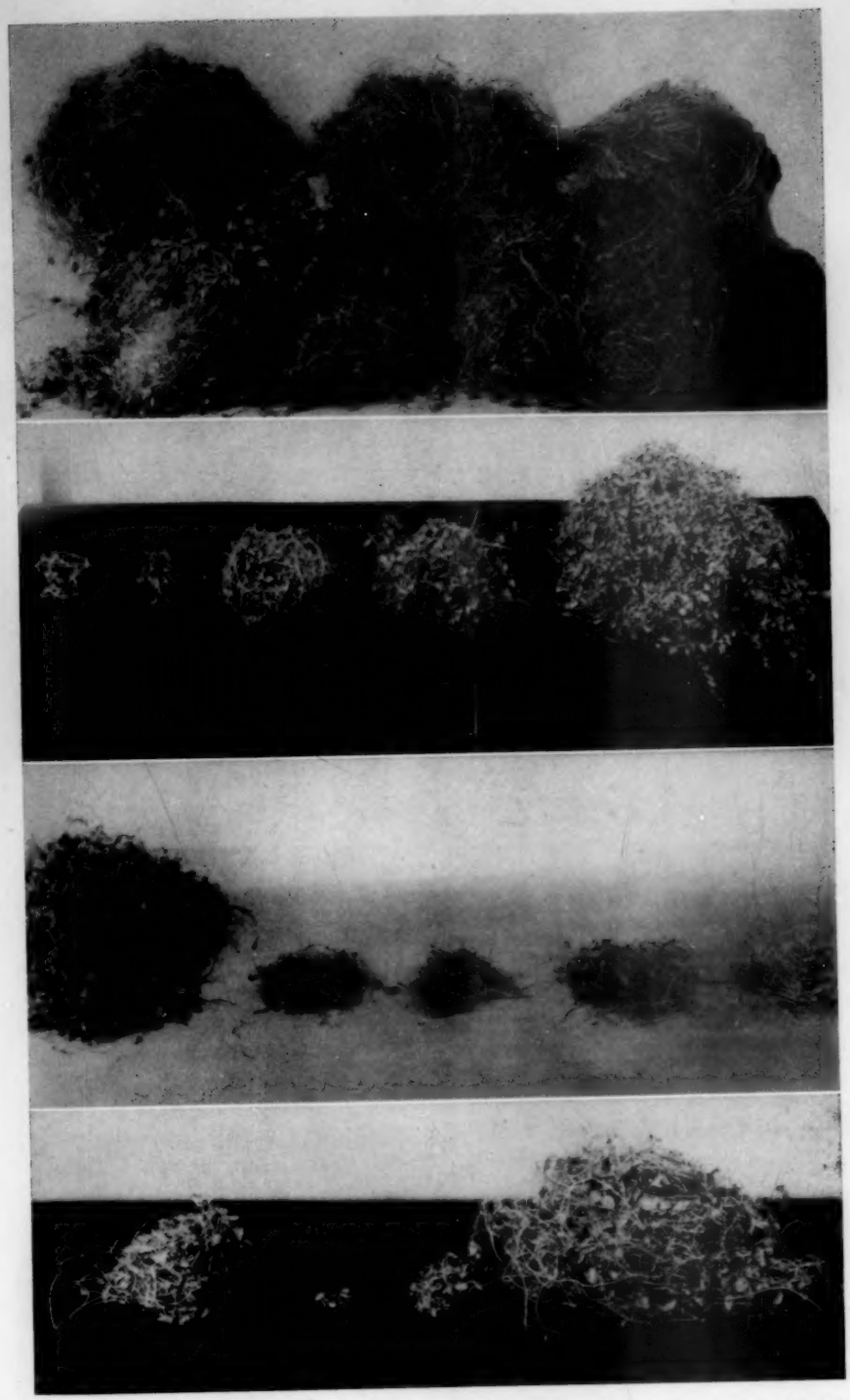

FIG. 1. Progressive depletion in the amount of aerial growth in European bindweed when subjected to reduced light (1937–40). Left to right: 1. Two layers of aster cloth (3,400 f.c.); 2. One layer muslin (1,050 f.c.); 3. One layer drill (650 f.c.); 4. Control; 5. Two layers black cloth (1 f.c.). Reading from top down: 1937, 1938, 1939, and 1940, respectively.

There was a general tendency for the moisture content of the roots to increase with the depth in the case of shelters No. 1 and 4. On account of the presence of rhizomes, the moisture content of the subterranean parts of the muslin and drill areas was higher at the first foot than at the third foot. The moisture content of the roots in the first foot of the unshaded area was the lowest of the entire series, but only slightly below No. 1 (two layers of aster cloth). The high moisture content of the roots of No. 2 (muslin) and No. 3 (drill) would suggest a highly succulent tissue compared with the control.

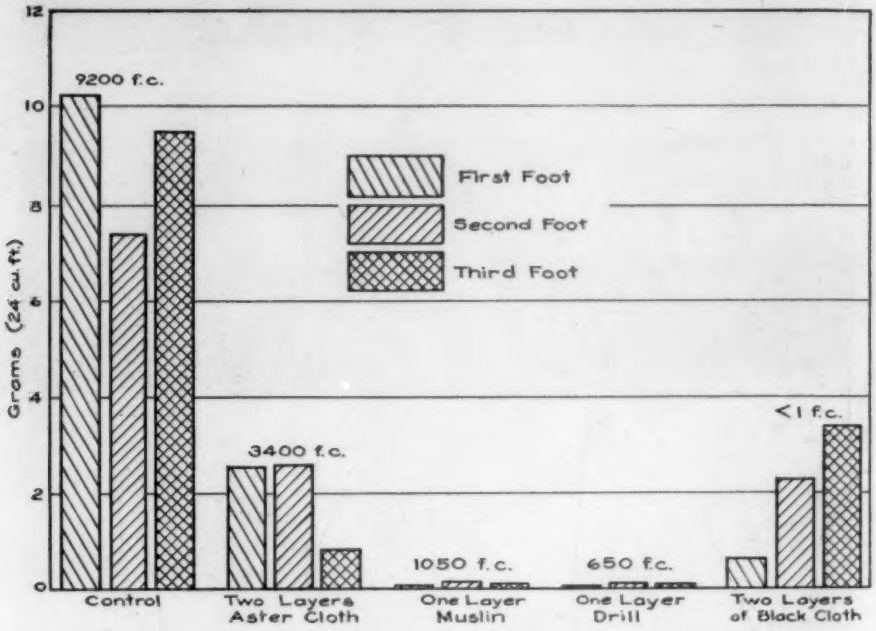

FIG. 2. Total available carbohydrates in the roots of the European bindweed at depths of one, two and three feet when the above-ground parts were subjected to different light intensities throughout the growing seasons 1937–1940.

The amount of root material in the control was considerably higher than that of any one of the cages followed in order by Nos. 4, 1, 2, and 3. It would be expected that the amount of roots taken from shelter 4 would be less than No. 3. The explanation given earlier for the greater amount of green tissue in No. 4 than in No. 3 will hold also for root growth. Sufficient allowance for lateral extension of the bindweed of the adjoining grain field had not been made. According to table IV, there were only 3 grams of green stem and leaf tissue at the end of the 1939 growing season, but 65.5 grams the next year. By calculation it was found that the dry weight per cubic foot at 1-, 2-, and 3-foot depths for shelters No. 1, 2, and 3 were: 0.108, 0.35, 0.091; 0.019, 0.031, 0.02; 0.006, 0.010, 0.006, respectively. Comparing these data with those from similar plots continuously cultivated as

reported by BAKKE *et al.* (**6**) it will be seen that low light intensity for three years was about as effective as intensive cultivation in reducing the quantity of roots.

The available carbohydrates measured in grams per 24 cu. ft. in the different habitats after four years of shading was least in the drill shelter,

TABLE V

THE EFFECT OF SHADING FOR 4 YEARS (1937–40) UPON THE AMOUNT OF ROOT MATERIAL AND ROOT RESERVES IN THE EUROPEAN BINDWEED TO DEPTHS OF 3 FEET (24 CU. FT. SOIL USED), OCT. 31, 1940

DESCRIPTION OF SHELTERS, STATUS OF GROWTH, LIGHT INTENSITY 5/21/40	DEPTH OF SAMPLE	MOISTURE	AMOUNT OF ROOT MATERIAL		CHEMICAL ANALYSIS			TOTAL AVAILABLE CARBOHYDRATES	AVAILABLE CARBOHYDRATES PER 24 CU. FT.
			GREEN WT.	DRY WT.	NITROGEN	TOTAL REDUCING SUGARS	GLUCOSE EQUIVALENT (STARCH)		
	ft.	%	*gm.*	*gm.*	%	%	%	%	*gm.*
No. 1—Two layers aster cloth. 3,400 f.c. 10 plants: 3 plants having shoots from crown.	1	71.88	9.71	2.59	2.31	10.18	15.31	25.49	0.602
	2	78.23	37.00	8.40	2.04	11.26	20.80	32.06	2.693
	3	79.64	10.75	2.19	1.84	11.23	24.71	35.94	0.787
No. 2—One layer muslin. 1,051 f.c. 6 plants: one plant having shoots from crown.	1	83.19	2.69	0.45	.....	13.05	8.62	21.67	0.097
	2	83.06	4.43	0.75	.....	9.73	8.42	18.15	0.136
	3	71.63	1.69	0.48	.....	7.67	5.85	13.52	0.065
No. 3—One layer drill. 600 f.c. 2 plants: no shoots from crown.	1	86.69	1.14	0.15	.....	9.30	11.72	21.02	0.032
	2	72.80	0.63	0.24	.....	4.59	23.50	28.09	0.067
	3	73.50	0.56	0.15	.....	21.50	12.60	34.10	0.051
No. 4—Two layers black cloth (cambric). Less than 1 f.c. No growth.	1	76.81	15.05	3.49	.....	14.95	23.70	38.65	1.35
	2	80.33	33.10	6.55	2.11	15.61	24.54	40.15	2.63
	3	84.10	58.00	9.80	2.45	14.49	20.89	35.38	3.47
No. 5—Control, no shading. 9,200 f.c. Ground covered with bindweed. 65 plants.	1	70.80	212.0	51.10	2.57	9.72	6.87	16.59	8.48
	2	77.68	202.0	47.40	2.33	7.72	9.79	17.51	8.30
	3	76.91	185.0	42.90	2.31	7.85	14.41	22.26	6.18

closely followed by the muslin-covered chamber. The reduction in the amount of available carbohydrates was greatest where the light intensity was lowest.

As the root material in shelters No. 2 and 3 was not sufficient for nitrogen determination even when micro methods were used, direct comparisons of the percentage of nitrogen could not be made. In the first two feet there was not much difference in the percentage of nitrogen present: in the third foot No. 1 (two layers of aster cloth), the percentage of nitrogen was somewhat lower

than in No. 5 ARNY (1) has stated that the nitrogen reserves even though present in relatively small amounts in perennial weeds are of great importance in the metabolism of the plant, particularly at the time of active early season growth. BAKKE *et al.* (5) have pointed out that the variation in the nitrogen fraction has little value as an index of regenerative power. FRAZIER (11) has stated that the total nitrogen fraction and the total readily available carbohydrate fractions are considered more reliable indexes than their component parts, the readily available carbohydrate fraction being the more reliable.

In the percentage of total reducing sugars in the roots of the European bindweeds grown under reduced light intensities there was not much difference between the control No. 5 and shelters No. 2 (muslin) and No. 3 (drill). In the muslin-covered cage in the second and third feet, the reducing sugars were low; the second foot of drill-covered cage (No. 3) was extremely low but the third foot had the highest percentage of total reducing sugars.

The percentage of starch in the roots of the plants grown in the muslin-covered cage (No. 2) was low throughout. The plants in the drill-covered cage followed the same general arrangement of its percentages of starch by being highest in the second foot. The control (No. 5) had the lowest percentage of starch, the two-layered aster cloth cage (No. 1) was next while No. 4 was the highest, throughout.

The total available carbohydrates followed much the same general plan of arrangement as given for starch.

Discussion

Sufficient evidence has been presented to show that a reduction in light intensity will reduce the amount of aerial and subterranean growth in a perennial plant such as the European bindweed. A reduction in the light intensity from 10,000 to 600 f.c. will materially affect the growth and development of the European bindweed and will exhaust the available carbohydrates to the point where there will not be sufficient food material for plant regeneration. A reduction in light intensity from 10,000 to about 1,000 f.c. for three consecutive years expended the aerial and subterranean growth. A light intensity of approximately 1,000 f.c. was about 3 to 4 times as effective in eliminating the root reserves as where two layers of aster cloth were used, having a light intensity of 3,400 f.c. (table V). No doubt a further reduction in light intensity would have brought on starvation earlier. It would seem as if a reduction of light to about 650 f.c. for three years would bring about an eradication of the European bindweed and be as effective as three years of intensive cultivation (5).

These facts should be useful in the practical control of European bindweed since it is well known that light intensity is reduced whenever a group of plants of the same species or other species are associated together. In thickly seeded crops like cane, sudan, millet, and soybeans, the reduction in light intensity may be such that an unwanted species like European bindweed will not be able to persist.

Summary

1. Five different habitats with varying light intensities were established by placing cloth covered chambers $4 \times 6 \times 4$ feet in an area heavily infested with European bindweed from July, 1937, until the end of the growing season of 1940.

2. The chambers with their maximum light intensities were as follows: (1) Two layers of aster cloth, 3,400 foot candles; (2) One layer muslin, 1,150 f.c.; (3) One layer drill, 650 f.c.; (4) Two layers black cloth (less than 1 f.c.); (5) Control, 10,000 f.c.

3. Daily light, temperatures, and evaporation readings were taken of three habitats during 1937.

4. Measurements of the thickness of the European bindweed leaves under different light intensities were as follows: (1) 3,400 f.c., 0.220 mm.; (2) 1,150 f.c., 0.210 mm.; (3) 650 f.c., 0.190 mm.; (4) 1 f.c., 0.160 mm.; (5) 10,000 f.c., 0.334 mm.

5. Measurements of the leaf area of the European bindweed were as follows: (1) 3,400 f.c., 8.15 sq. cm.; (2) 1,150 f.c., 7.15 sq. cm.; (3) 650 f.c., 3.76 sq. cm.; (4) 1 f.c., 0.69 sq. cm.; (5) 10,000 f.c., 3.21 sq. cm.

6. The shelter covered with drill produced at the end of the 1937 season, 1,418.5 grams of green bindweed material. At the end of 1940 season there was 0.7 gram present.

7. The average amount of root material per 24 cu. ft. at the end of 1940 for the first 3 feet for the control (10,000 f.c.) was 200 grams (green weight); percentage total available carbohydrates, 18.79; amount of total available carbohydrates, 7.65 grams (dry weight). For the drill covered area (650 f.c.) the average amount of root material for the first 3 feet was 0.74 grams; percentage total available carbohydrates, 27.74; total carbohydrates, 0.050 grams.

8. A successful smother or competitive crop when used to eradicate the European bindweed is fundamentally a process of light restriction. Sufficient shade must be produced early in the development of the plant which predominates.

IOWA AGRICULTURAL EXPERIMENT STATION
AMES, IOWA

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A SIMPLIFIED RECORDING POTENTIOMETER

R. H. WALLACE

(WITH SIX FIGURES)

Some years ago (1) the author described a recording potentiometer designed primarily to meet the requirements of flexibility so necessary in physiological work. A number of these instruments were built and are still in use. Ever since this earlier instrument was developed the writer has continued to improve and simplify until now one which is far superior in all respects to this first instrument has been constructed. This instrument is fundamentally a self-balancing potentiometer controlled by radio tubes. It differs from the earlier model, however, in being much more sensitive, stable, compact, and particularly mechanically reliable. All parts used in its construction are standard and only a minimum amount of machining is necessary.

The bridge scheme

The increased simplicity of the new instrument is evident from its description and mode of operation. In figure 1 is given the schematic diagram of the bridge circuit. It will be noted that there is a fixed slide wire bridge

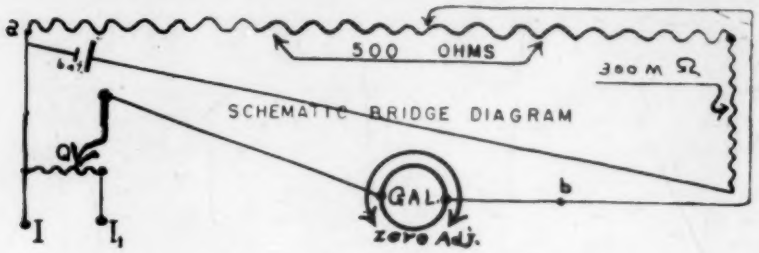

FIG. 1. Schematic diagram of bridge.

of about 500 ohms connected in series with a fixed resistance of 300,000 ohms. The two free ends of this resistance combination are connected to the two terminals of an ordinary 1.5-volt (1,500-millivolt) flashlight dry cell. There is, therefore, across the slide wire bridge 500/300,000, or 1/600 of 1,500 millivolts, or 2.5 millivolts. The bridge has this fixed millivoltage value at all times. It will be noted in the figure that the traveling arm of the instrument is connected in series with a galvanometer and over to the input I_1. If one now connects a potential between the inputs I and I_1 the galvanometer will deflect either to the right or the left unless the contact arm of the bridge is just at the point of balance.

Adjustment of the zero for any position on the bridge is obtained by turning the cap of the galvanometer. Thus zero is controlled by the simple device of bucking the potential on the bridge by a torsion of the spring suspension of the galvanometer. This arrangement gives three valuable char-

acteristics. In the first place, it eliminates one battery and two resistances used in the earlier instrument to get the same control. Secondly, it gives one an excellent check on the condition of the battery, for any variation of its output shows up immediately as a drift of zero. Thirdly, since a mechanical force is bucking an electrical force, any instability from thermocouple effects in the bridge circuit will also show up as a drift of zero.

Referring again to figure 1, it is seen that any unknown between I and I_1 must not exceed $2\frac{1}{2}$ millivolts. If, however, one connects an additional resistance (Q) across inputs I and I_1 and then connects the I end of this resistance to the "a" end of the bridge and connects the variable arm to the galvanometer, an unknown of any potential may be measured. It should be noted, however, that adjustment of the variable arm varies the resistance in series with the galvanometer and, consequently, changes the zero point slightly. It is necessary, therefore, to first obtain the correct millimeter deflection by adjustment of this arm and then to set the zero at the position desired by adjustment of the galvanometer cap. We thus have a bridge universal as to position of zero and millivoltage value.

The exact values of the slide wire bridge and its supplementary resistance are not critical because the calibration of the instrument is always made by connecting directly to resistance Q. Calibrations can be in electrical terms with standard potentiometers, in millimeters on recording drum per degree for thermocouples, in foot-candles per millimeter when measuring light, or any other similar system.

A very small (finger size) dry cell is adequate to give the bridge voltage because it is in series with a 300,000-ohm resistance. In fact, recently a battery two years in service still gave good zeros. The current drain of the circuit is so minute, 5 micro-amperes, that one gets shelf life from the battery.

The activating mechanism

The activating mechanism used in this device is shown schematically in figure 2 and pictorially in figures 3–6. It will be noted that three fundamental electrical parts are involved in operating the activating device (fig. 2). These are a reversible motor, two radio tubes, and two photocells together with transformers, batteries, etc., to make these parts function.

Perhaps the most critical of these parts is the motor (M) for it was not until this motor was found that the present simplification of the earlier instrument became possible. This motor (Type PYAZ Barcol Motor, Barber-Colman, Rockford, Ill.) is a small reversible induction motor with a 450 to 1 gear reduction built into the case. The starting mechanism of this motor consists of two built-in transformers. When the leads to one transformer are shorted, current flows through it and the motor runs in one direction. When the leads to the other transformer are shorted, current flows through it and the motor turns in the opposite direction. If both transformers have current flowing at the same time, the motor will either stall or move slowly in the direction of the transformer passing the stronger

current. Even though both transformers are passing full current, the motor is so designed that it does not overheat and no damage whatever results. These electrical characteristics are extremely important; they eliminate the necessity for limit switches on the instrument, and render impossible any damage from attempted activation of the motor in two directions at one time.

It will be seen that the starting transformers are connected to the plates of the two radio tubes, while their common junction is connected to the cathodes of the same two tubes via the 110-volt source (fig. 2). The resistance *R* in series with this common connection prevents excessive current flowing through the tubes and motor. The tubes in this scheme essentially substitute for the shorting of the leads of the transformers mentioned above in that they allow current to flow, thus activating the motor in one direction or the other, depending on the grid charge within each tube.

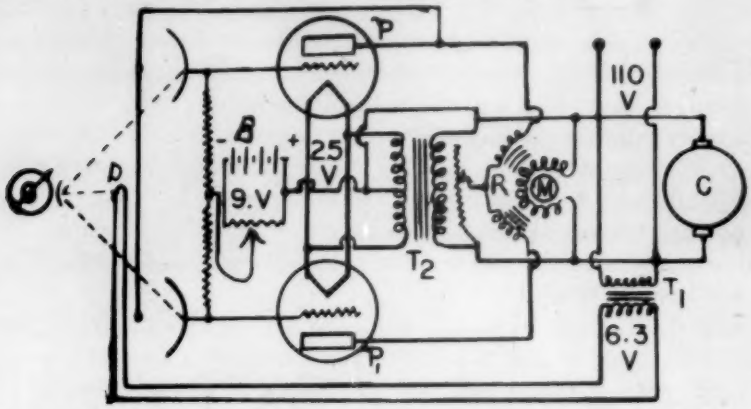

FIG. 2. Wiring diagram of activating unit showing connections for clock, pilot bulb, thyratrons, etc.

The tubes used are small gas-filled thyratrons, the common 2A4G of any radio store. These tubes, being gas-filled, are unaffected in their sensitivity by the ambient air temperature; they, therefore, give better control than the mercury-filled tubes formerly used. These tubes fire continuously with floating grid or zero bias. For this reason, to prevent them from firing, a small battery (*B*) is connected as shown in figure 2 between the cathode and the grid with suitable grid leaks.

Several types of photocells have been used, but the one which seems best suited to this circuit is the R.C.A. 922. This photocell is a vacuum type, highly sensitive to the red end of the spectrum, and is able to stand rather high voltages without injury. These characteristics suit it well to this circuit because the pilot bulb *D* is also high in red. In addition, since these tubes can stand high voltages, one can tap off directly from the AC source as a plate supply, and thus eliminate a battery for the photocells. This is done by connecting directly to the AC from one of the starting transformers (fig. 2). A lighted photocell, then, functions as follows: On each plus cycle of

the AC, current passes from pin to shield; this makes the grid of the thyratron plus, which allows current to flow from plate to cathode in the starter transformer circuit, thus causing the motor to run.

The motor has two small transformers built within itself as starting mechanisms. These transformers have only 20-volt outputs, and since the voltage drop within the 2A4G tube is 16 volts, it is impossible to operate the scheme directly on this small voltage. However, since the cathode of the tubes is already tied directly to one side of the 110-volt line, it is very simple to connect the common terminal of the two starting transformers to the other side of this line by way of a limiting resistance (R), and thus get either 90 or 130 volts between cathode and plate of the 2A4G tubes, depending upon whether the transformers are in or out of phase with the 110-volt line. For our purposes, either 90 or 130 volts works equally well.

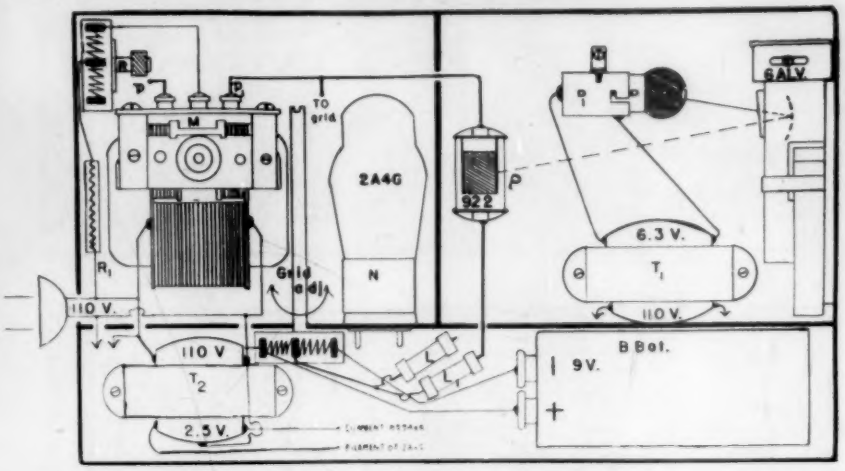

FIG. 3. Vertical longitudinal section through instrument showing spacing, shape, and arrangement of parts.

The pictorial arrangement of parts (figs. 3 to 6) shows that the scheme functions as follows: Light from pilot bulb (D) strikes the mirror in the galvanometer (G) and is thrown back into photocell (P). Photocell P, as mentioned above, puts a plus charge on the tube 2A4G. This tube then allows current to flow through a shading transformer and the motor runs. Tube 2A4G is protected from excessive current by the set resistance $(R)_1$ mentioned above. Control of the speed of the motor (M) is obtained by adjustment of the variable resistance (R) in series with this fixed one (figs. 3, 4).

In actual operation, the beam of light reflected back from the galvanometer mirror lights both photocells in part, so that both 2A4G tubes are partially activated. Ordinarily, when near balance, these two tubes flicker. When firing steadily they show a rich lavender discharge. This discharge is very valuable in showing what the instrument is doing. After one is

acquainted with it, it is possible to determine instantly that everything is working correctly by merely watching the alternate flickering of the two tubes, or the steady glow.

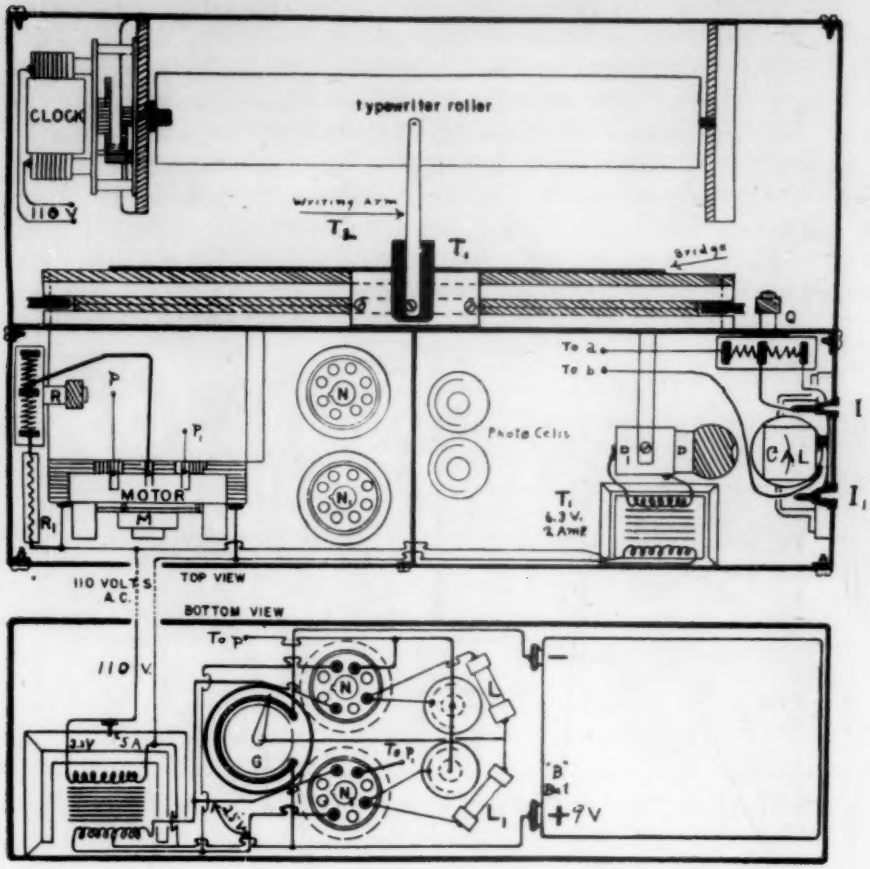

FIG. 4. Top view showing spacing of parts. Lower part of diagram shows wiring connections of these parts as seen from below.

Mechanical construction

In the present instrument the mechanical construction is simple and very reliable. This improvement is primarily due to the motor mentioned above. This motor has built within itself a 450 to 1 gear reduction. Consequently, a powerful torque is delivered to the power shaft. The mechanism to which this motor is attached consists essentially of two parts, a sliding mechanism with its track and three pulleys for moving the slider (figs. 4, 5, 6). In figure 5 is shown the end view of the sliding mechanism. Although this can be individually constructed if desired, material such as shown in the figure, accurate to a few thousandths of an inch, is commercially available and serves the purposes very well. At each end of this dove-tailed brass track

is mounted a small pulley. A third pulley is mounted on the power shaft of the motor. The track is mounted on a metal panel (the heavy black line in figures 4 and 5). A three-inch hole (fig. 6) is cut in this panel to receive the face of the motor. The motor is mounted on the opposite side of this same panel and clamped in place by four screws that are present in its gear box. This places the third pulley beneath the two pulleys at the ends of the brass track. An ordinary piece of surgeon's catgut is then attached to one end of the brass slider, passed over the adjacent pulley, looped around the motor pulley, then up over the third pulley, and is attached to the oppo-

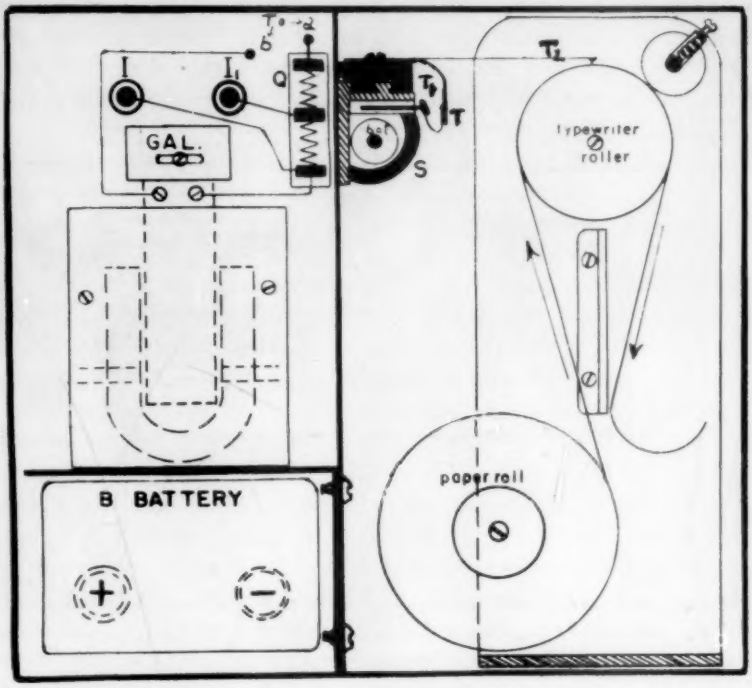

FIG. 5. End view showing details of sliding mechanism, bridge, galvanometer connections, and paper carriage.

site end of the brass slider. The power shaft of the motor moves 3 r.p.m. By selecting a suitable size of power pulley, one can obtain almost any speed of travel desired. A speed well suited for most purposes is full scale in 10–20 seconds. This slider device eliminates the limit switches because, when the slider reaches the limit of its run, it merely contacts the pulley and the catgut slips on the power pulley. The brass track also gives a simple and effective means of mounting the slide-wire bridge. It will be noted (fig. 5) that the resistance strip is merely mounted in a nonconducting material and then the whole mounted on the under face of the dove-tailed slider track. It is held in place by two small screws at the ends. The connections for the bridge are shown in figure 6. Since the wire on the resistance strip

will give thermocouple effects with copper, one must so design the system as to render these harmless. This is accomplished by unwinding, from each end of the stripping, wire sufficient to reach to the set resistance and the connection "*a*." The set resistance is soldered directly to the bottom of the bridge battery while the other end of this battery is attached back to connection "*a*." It is likewise necessary to unwind about two feet of this resistance wire from the resistance strip and make up the cable which runs from *T* (traveling arm) to connection "*b*." In this manner all unlike metal junctions are in close proximity beneath the brass slider track and ordinarily cause little trouble. However, as an additional precaution, a thermal shield (*S*) is mounted under the brass track covering the battery and connections "*a*" and "*b*" (fig. 5). The two copper wires attached at "*a*" and "*b*" lead through the panel and connect to the galvanometer and resistance *Q*, thus completing the bridge wiring (fig. 1).

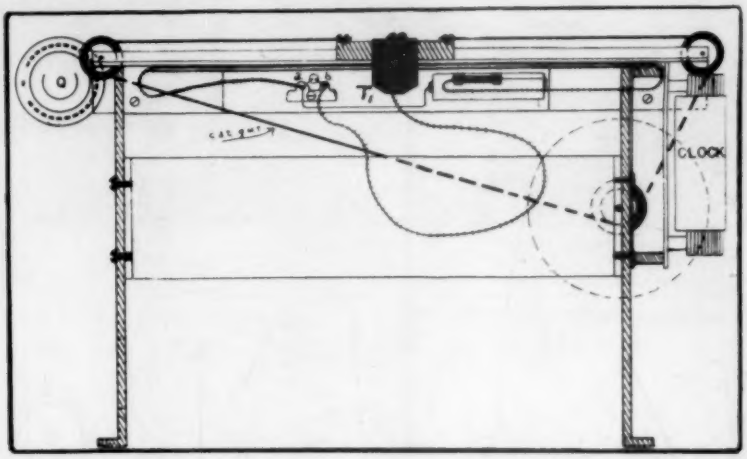

FIG. 6. Front view showing pulley arrangements, sliding mechanism, and special wiring precautions to avoid thermal effects. Resistance wire shown in cross-hatched line.

The traveling contact (*T*) is obtained by mounting a heavy brass piece (T_1) directly on the brass slider by means of a screw (fig. 5). This same screw likewise holds the writing arm (T_2). If one solders a flexible bronze spring to the heavy brass piece, it serves well to maintain contact on the resistance bridge. However, since the traveling contact (*T*) of the resistance cable mentioned above must be insulated from ground, it is necessary to wrap the end of this flexible spring with adhesive tape, and then wrap twenty or thirty turns of the resistance wire over this for the contact itself. This resistance wire takes solder very well, and it is easy to make a smooth sliding contact.

The paper carriage (figs. 4, 5, 6) is made with two vertical uprights held together by one spanner. Between these uprights are mounted three rollers. The lower one of these is a wooden mandrel to carry the roll of paper and is held in place by two wood screws. The second roller, turned by a small

electric clock (C) mounted at the end, constitutes the writing surface, and can be any size depending on the speed of travel one desires. The third, small roller is an idler to hold the paper in contact. Clocks of almost any speed are available from 1 r.p.m. to one revolution per week.

The correct spacing of the galvanometer, pilot bulb, and photocells is shown in figures 3, 4, and 5. This potentiometer fits in a standard metal radio cabinet $7\frac{1}{2} \times 8 \times 12$ inches. Its total weight is 22 lbs. The cost of all materials for its construction is about $50.00.

Operation of the instrument

The recorder is a delicate automatic potentiometer in which a modified Wheatstone bridge is held on balance by means of a mechanical system controlled by radio tubes. There is no connection between the bridge circuit and the electrically-controlled balancing circuit other than the reflected beam of light of the galvanometer. In actual operation the instrument functions somewhat as follows: The traveling arm of the bridge is out of balance with the unknown which causes the beam of light to fall on one of the photocells. This activates in full the 2A4G tube which causes the motor to run. As the null point is approached, sufficient light strikes the other photocell to make the second tube activate intermittently and flicker. Shortly thereafter, both tubes flicker, one more rapidly than the other. At complete balance, both tubes glow with a dull glow interspersed with flickering.

This instrument can be used equally well as a recording millivoltmeter or micro-ammeter. It will be noted (fig. 1) that, whatever the millivoltage placed across Q, it can be made to cover any part or all of the scale by adjustment of this resistance. And, likewise, zero can be placed at any point on the scale. One can thus set the sensitivity to give any desired deflection for the unit under measure. The instrument works equally well as a recording micro-ammeter. It has been used several years to record light intensity. In this work vacuum tube photocells located on a roof have served as the light-sensitive unit. These cells, although rated at 20 to 40 microamperes, are so filtered as to give less than 1 micro-ampere for full sunlight at noontime. This low drain insures extremely long life for the sampling unit. This work will be described in detail in a subsequent paper. The very small current mentioned above is passed through resistance Q and measured as a voltage drop across it. The variable arm is adjusted to give the desired foot-candle value per mm. This instrument has been used to measure temperature with a thermocouple, light with vacuum tube photocells, humidity with a wet-dry junction thermocouple, and the usual potentiometric measurements in hydrogen ion, electro-metric titration, and polarigraphic analysis.

During the development of this instrument some interesting difficulties were encountered in stabilizing for low bridge values. As ordinarily run at 2.5 millivolts for the full 200-mm. scale, the zero usually stayed within 1 mm.; at times, however, serious drifts of 5 or more mm. occurred. This

difficulty was finally eliminated by selecting carefully matched thyratron tubes. The tubes must be matched in the instrument itself. The method of matching the tubes is too extended to give here but will be found in the blueprints mentioned below. With well matched tubes this instrument will maintain a zero within 1 mm. for a line voltage variation from 95 to 125 volts.

Full scale bridge values as low as 0.08 millivolt (80 microvolts) have been tested and under favorable conditions have proved satisfactory. It is not recommended, however, that these very low values be used except on controlled line voltage and with more adequate thermal shielding than in the present instrument. Thermal instabilities can be very minute and still give serious drifts when the bridge is 80 microvolts. For with this value 1° C. with a copper Constantine thermocouple gives about 110 mm. deflection. Full sized blueprints, together with detailed specifications, and instructions for making the recorder are available from the author.

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SOME EFFECTS OF BORON AND MANGANESE ON THE QUALITY OF BEETS AND TOMATOES[1]

OREN B. GUM, H. D. BROWN, AND R. C. BURRELL

(WITH TWO FIGURES)

Introduction

In a series of greenhouse experiments extending from June, 1941, to June, 1944, inclusive, beets (*Beta vulgaris*) and tomatoes (*Lycopersicon esculentum*) were grown in media of controlled composition to determine what, if any, effect a deficiency of boron or manganese might have on quality which was measured by the amounts of moisture, sugar, alcohol soluble and insoluble nitrogen, carotene, ascorbic acid, thiamin, and riboflavin that were present.

Boron was selected for study because it is an essential element for most if not all of the important crop plants (**3, 7**). Symptoms of boron deficiency can be easily recognized (**4**) and the many reports in the literature indicate that there is a rather widespread deficiency of this element; soils lacking boron adequate for good crop growth have been reported in over half of the forty-eight states (**11**) with noticeably large areas along the Atlantic seaboard, in the Great Lakes region, and in the Pacific Northwest. A very good list of important papers which deal with boron in plants and substrates is appended to the paper by PARKS, LYON, and HOOD (**10**).

Manganese was selected for further study for reasons somewhat similar to those enumerated for boron, but especially because of the report by HESTER (**5, 6**) that this element seemed to have a definite effect on the vitamin C content of the tomato.

Experimental methods

Four large concrete beds, each holding about 150 gallons of solution, and two smaller beds were available for hydroponic studies. Gravel beds were also used. The beds were thoroughly cleaned and asphalted to minimize contamination; when gravel was used as a medium, it was thoroughly washed. Boards were used to cover the hydroponic beds, and holes were conveniently placed in these boards so that 4-inch pots could be inserted. It was thought that using these beds rather than individual pots would approach more nearly conditions encountered in the field. For the same reason it was not planned to obtain absolute deficiencies, but merely different levels of the element in question. With tomatoes the hydroponic method proved especially suitable; with beets, gravel culture was preferable. At first some difficulty was encountered in producing deficiency symptoms in gravel cultures because of a contamination of the materials used with a

[1] This study was made possible by a fellowship which was supported by grants from The Tennessee Corporation during the period 1941–44.

TABLE I

CULTURE SOLUTION; MAJOR ELEMENTS

COMPOUNDS	GRAMS PER 10 GALLONS	CONCENTRATION OF ELEMENT				
		N	P	K	Ca	Mg
		p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
$Ca(NO_3)_2 \cdot 4H_2O$	22.2	100	.....	.....	143	.....
$Ca(H_2PO_4)_2 \cdot 2H_2O$	11.5	.....	75	.....	49	.....
KCl	29.5	.....	.....	200	.....	.....
$MgSO_4$	9.2	.....	.....	.....	.....	50
Total	.....	100	75	200	192	50

sufficient quantity of the trace element to produce good growth. By growing several successive crops in the same media, however, boron became depleted and deficiency symptoms occurred. It proved to be impossible to obtain well defined manganese deficiency symptoms in the large beds. So another series of experiments was inaugurated in which, in order better to control the composition of the substrate, 4-liter beakers were used for individual plant experiments.

All chemicals used were of C.P. quality. For the first experiments distilled water was used but in the subsequent experiments, tap water was found to be just as satisfactory for the boron deficiency experiments. For managanese deficiency, on the other hand, even the distilled water was not suitable and finally repurified chemicals and triple distilled water had to be used and the experiments had to be carried out in large beakers as stated above. Table I gives the composition of the culture solution in terms of the major elements and the salts used to supply them.

The chemicals containing the major elements were weighed, dissolved in a minimum amount of water, and added to the water in the beds or containers. The appropriate minor elements were added from stock solutions whose compositions are given in table II.

For the complete solution, sufficient of each of these stock solutions was added to bring the concentration of Fe to approximately 2.0 p.p.m.; Mn, 0.05; B, 1.0; Cu, 0.05; and Zn, 0.005 p.p.m.

TABLE II

MINOR ELEMENT SUPPLEMENTS

COMPOUND	GRAMS PER LITER	CONCENTRATION OF ELEMENT	ONE CC. OF STOCK SOLUTION IN 1 GAL. OF SOLUTION GIVES
	gm.	*p.p.m.*	*p.p.m.*
$MnSO_4 \cdot 4H_2O$	15.3	1892	0.5 Mn
H_3BO_3	23.0	3786	1.0 B
$CuSO_4 \cdot 5H_2O$	0.75	189	0.05 Cu
$ZnSO_4 \cdot 7H_2O$	0.85	189	0.05 Zn
Ferric citrate	20.0	3785	1.0 Fe

The concentrations of the elements were checked by frequent analyses of the culture solutions. Sufficient quantities of the salts or, for the minor elements, the stock solutions were then added to replace the amounts that had been removed by the growth of the plants. In certain series no boron or manganese was added, all that was present being due to the small amounts from the salts, water, or gravel. In other series supplemental manganese and boron were added intermediate to the quantities used for the complete solution and in several series amounts greater than these were used in order to produce excess symptoms. The latter, in general, resulted in the death of the plants and were not followed by chemical analysis.

The tomato was a strain developed at the Ohio State University; the beets were of the Detroit Dark Red Variety. In the gravel beds the seeds were germinated directly on buried cheesecloth or blotting paper. For the hydroponic beds the tomato plants were first grown in soil which was later removed from the roots by washing. They were then transferred to the pot supports.

Analytical methods

The method for determining the amount of boron in the culture solutions and in the plants was a modification of the one described by NAFTEL (**9**) which depends upon the development of a cherry-red color with an alcoholic solution of turmeric. The procedure differed from that of NAFTEL in the ignition of the nitrates and the use of a spectrophotometer and appropriate calibration curve for percentage transmittance, instead of an electrophotometer. Especially in the determination of boron in the culture solutions, nitrates were found to interfere with the full development of color and their removal by ignition of the evaporated residue was necessary. The maximum amount of absorption was found to be at 530 mμ and working curves produced at this wave length were found to obey Beer's Law up to 7 micrograms. The boron in the plant material was extracted by treating the ash with normal hydrochloric acid, filtering, washing the residue thoroughly with hot water, and diluting to a convenient volume.

Manganese was determined by oxidizing to $KMnO_4$ and measuring the intensity of the resulting color by use of a spectrophotometer. At an absorption wave length of 520 mμ Beer's Law was valid up to a concentration of 20 micrograms. In preparing the sample, an aliquot of a solution or a plant ash sample was taken which contained 1 to 20 micrograms of manganese. There was added 15 cc. of concentrated H_2SO_4 and 5 cc. of concentrated HNO_3 and finally triple distilled water to make a volume of approximately 75 cc. After mixing, the solution was heated to boiling and 0.3 gm. of potassium iodate was added in small quantities and heating was continued until no further increase in the intensity of the color was noted. After cooling and diluting to volume the percentage transmittance was measured.

Nitrates in the culture solutions were determined by the diphenylamine test. Potassium was determined by the nephelometric determination of the density of the potassium cobaltinitrite precipitate; calcium, by the amount

of the calcium oxalate precipitate; magnesium, by the use of Titan Yellow in the presence of alkali; phosphorus, by the measurement of the molybdate blue color with a spectrophotometer; and iron, by a reagent that contained both ferro- and ferricyanide in order to measure both ferrous and ferric ions. All these methods are in common use in hydroponic experimentation, except possibly the measurement of the phosphorus by means of a spectrophotometer instead of a visual colorimeter.

Leaves from the same part of the plant were removed from as many different plants as possible to yield considerably more material than was necessary for analysis. This material was chopped or ground and mixed thoroughly to insure a representative sample. Likewise as many tomato fruits or beet roots were sampled as were available. The prepared sample was spread out on a large plate and "quartered" to give the sample for analysis. These aliquots were extracted with boiling 80 per cent. ethyl alcohol (**2, 8**) diluted to a definite volume and aliquots of these extracts were used for the various quality tests cited below with the exception of vitamins; for these determinations the fresh samples were used directly. Some of the original ground material was dried and ashed to yield samples for mineral analysis.

Dry matter was found by evaporating 25-cc. aliquots of the alcoholic extract and taking into account the weight of the dried residue from the alcoholic extraction.

Reducing and total sugars were determined by evaporation of aliquots of the alcoholic extract (200 cc. usually), taking up with water, clarifying, and de-leading (**2**) followed by the Shaffer-Hartmann procedure (**12**). For total sugar, inversion was carried out by use of invertase (**2**).

Alcohol-soluble nitrogen was determined by preliminary reduction with Devarda's alloy followed by the Kjeldahl procedure; and insoluble nitrogen, by Kjeldahl analysis of one-gram samples of the dried, insoluble residue.

Carotene was determined on fresh five-gram samples according to the tentative procedure described in "Official Methods" (**2**). Vitamins B_1 and riboflavin were determined by methods described in "Cereal Laboratory Methods" (**1**). The method of ROE and KUETHER as modified by SHETLAR (**12**) was used for ascorbic acid.

Experimental results

BORON-DEFICIENT BEETS

While the leaves of the control beets remained green, those of the boron-deficient plants changed to a dark red color. Soon the stem apices of the beets in the boron-deficient bed began to wilt and die. When harvested, the control beets were smooth and of good appearance while the boron-deficient beets showed signs of severe injury. Cankers with necrotic areas were present on all of the tap roots and in the most extreme instances completely encircled them (fig. 1).

FIG. 1. Control (left) and three boron-deficient beets.

BORON-DEFICIENT TOMATOES

The plants were grown in beds with aqueous media either lacking adequate boron or containing sufficient for favorable growth. From plants in the control bed fruits of good appearance were obtained and the foliage was vigorous. The boron-deficient tomato plants were smaller than the controls during the early part of their growth. Later they became more nearly alike, although the stems of the boron-deficient plants were ultimately somewhat smaller than those of the controls. Just prior to fruiting the stems of the boron-deficient plants became very brittle and considerable care had to be exercised to avoid breaking the tops of the plants when handling them. This condition was evident before any symptoms of boron deficiency were observed in the foliage. The next symptom observed was that the leaves

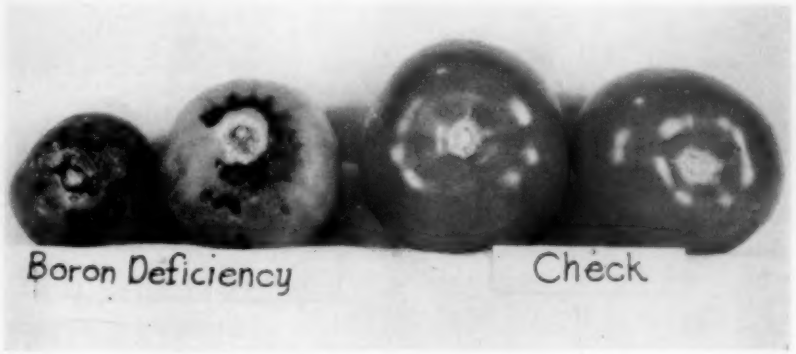

FIG. 2. Boron-deficient tomatoes and control.

toward the tops of the plants became brown along the edges, especially near the apices, and the veins assumed a pinkish tinge. The fruits which had set by this time developed brown necrotic spots particularly abundant near the stem end. These brown spots did not appreciably increase in size as the tomatoes ripened. Such fruits did not develop a full red color (fig. 2). At this stage the entire plant had taken on a deadened appearance, the tips being most affected.

Manganese-deficient tomatoes

No well defined symptoms of manganese deficiency were present in tomato foliage from plants that were grown in the large beds by hydroponic culture. Toward maturity of the plants, after the fruits had set, a chlorotic condition was usually present in the youngest leaves which resembled mild deficiency symptoms. Plants showing unmistakably definite symptoms were grown in substrates of purified chemicals in 4-liter beakers. The leaves toward the tops of the plants first showed a fading of the green to a yellow color between the veins of the leaves. This yellow color became more marked and spread until nearly all of the area between the veins was involved. The veins remained green for some time and this produced a characteristic mottled chlorosis.

Discussion

Very little difference in composition was produced by varying the quantities of the minor element between no addition up to the level used in the complete solution. So in general just two types of samples were analyzed: those from treatments in which none of the trace element was supplied; and those from plants that were grown with complete solutions, viz., the controls. The results of a great many of these analyses are presented in table III.

The boron-deficient tomato fruits contained less dry matter than did the controls; this was also true of the boron-deficient beets as compared with the controls. The percentage of dry matter in the tomatoes from the control bed was somewhat greater than that of the manganese-deficient tomatoes. The manganese-deficient beet roots, however, contained more dry matter than the controls.

Neither boron nor manganese deficiency had any very great effect upon the amount of alcohol-soluble nitrogen in tomato fruits. There was some diminution of insoluble nitrogen. With beet roots, however, with both boron and manganese deficiency there was a very considerable increase in alcohol-soluble nitrogen and insoluble nitrogen. Subsequent analysis of the boron-deficient beets showed that they contained nearly twice as much amide-ammonia nitrogen as the controls. No nitrates were found in the boron-deficient beets while the amounts present in the controls was considerable. Other investigators have reported similar changes in the soluble nitrogen fraction of boron-deficient plants (**4**).

The amounts of reducing and total sugars were less in both boron- and manganese-deficient tomato fruits than in the controls. With beets the same sort of a relationship held true for total sugars but not for reducing sugars.

TABLE III

COMPOSITION OF DEFICIENT AND CONTROL TOMATOES AND BEETS REPORTED AS PERCENTAGES OF THE FRESH WEIGHTS WITH THEIR STANDARD ERRORS (13)*

	Boron-deficient	Manganese-deficient	Control
Dry matter			
Tomato (fruit)	6.08 ±0.64 (4)	5.54 ±0.75 (9)	7.75 (2)
Beet (roots)	13.22 ±0.61 (3)	16.44 ±1.20 (4)	14.29 (1)
Reducing sugars			
Tomato (fruit)	1.44 ±0.315 (4)	1.21 ±0.245 (9)	1.89 (2)
Beet (roots)	0.39 ±0.06 (3)	0.35 ±0.038 (4)	0.20 (1)
Total sugars			
Tomato (fruit)	3.16 ±0.53 (4)	2.56 ±0.54 (9)	4.02 (2)
Beet (roots)	6.88 ±0.655 (3)	7.57 ±1.10 (3)	8.96 (1)
Alcohol-soluble nitrogen			
Tomato (fruit)	0.085 ±0.015 (4)	0.093 ±0.013 (9)	0.094 (2)
Beet (roots)	0.254 ±0.011 (3)	0.167 ±0.007 (4)	0.139 (1)
Insoluble nitrogen			
Tomato (fruit)	0.071 ±0.004 (4)	0.056 ±0.006 (9)	0.080 (2)
Beet (roots)	0.193 ±0.022 (3)	0.220 ±0.025 (9)	0.070 (1)

* Duplicate analyses were made on material from each replication and the number of replications is indicated in parentheses. The control values are averages of several analyses of the number of replications indicated.

TABLE IV

VITAMIN, BORON AND MANGANESE CONTENTS OF TOMATOES AND BEETS

Crop	Sample	Previous treatment	Ascorbic acid*	Carotene†	Thiamin†	Riboflavin†	Boron‡	Manganese‡
			mg.	γ	γ	γ	*p.p.m.*	*p.p.m.*
Tomato	Foliage	Boron-deficient	0.34	93	Trace	0.58	6.8	.....
"	"	Control	0.32	95	"	0.68	24.4	.....
"	Fruit	Boron-deficient	0.35	nil	nil	nil	.....	.....
"	"	Control	0.44	118	"	0.67	.....	.....
"	Foliage	Manganese-deficient	0.30	99	Trace	0.52	.....	Trace
"	"	Control	0.34	118	"	0.67	.....	32
"	Fruit	Manganese-deficient	0.44	116	nil	0.59	.....	.....
"	"	Control	0.44	118	"	0.67	.....	.....
Beet	Foliage	Boron-deficient	0.031	32	Trace	1.44	.....	.....
"	"	Control	0.063	119	"	1.76	.....	.....
"	Roots	Boron-deficient	0.088	nil	.....	0.67	2.0	.....
"	"	Control	0.088	"	.....	0.96	2.0	.....

* Milligrams per gram fresh weight.
† Micrograms per gram fresh weight.
‡ Parts per million of oven dried weight.

The ascorbic acid contents (table IV) of neither the foliage nor the fruit of the tomatoes showed any consistent differences with manganese treatments (**6**). The ascorbic acid content of the boron control beet leaves was greater than that of the deficient leaves.

In general the smaller the quantity of manganese in the tomato foliage, the less carotene it contained. Beet leaves showing symptoms of boron deficiency contained much less carotene than normal-appearing leaves from the same plant. The amount of carotene in such normal-appearing leaves from boron-deficient plants was comparable to that present in the leaves of the controls.

Controls contained slightly more vitamin B_2 (riboflavin) than did boron- or manganese-deficient beets and tomatoes. Only a trace of vitamin B_1 was found in either the tomatoes or the beets.

The amount of boron found in the foliage varied directly with the amount of boron available to the plant. Symptoms of boron deficiency were associated with the presence of small amounts of boron in the top portions of the deficient plants as compared with the controls. There was much less difference in the amount of boron present in the roots of deficient as compared with control plants.

The amount of manganese found in the plant material varied directly with the amount of manganese which was present in the substrate in which the tomatoes were grown. This is well illustrated by the amounts of manganese found present in the manganese-deficient, low-manganese, and optimum-manganese tomato foliage. In two different series of crops the manganese increased from a trace in the foliage of plants grown in the manganese-deficient solutions to 32 and 60 p.p.m. in the foliage from plants whose culture solutions contained increasing levels of manganese. The concentration of manganese in foliage from younger plants is greater than that from older plants. The manganese content of beet or tomato foliage must be extremely low before symptoms of manganese deficiency are evident.

A taste test was designed to determine if there were any noticeable differences in the flavor and structure of tomato fruits from different manganese treatments. Tomatoes from the manganese-deficient, low-manganese, and favorable-manganese plots were sampled by 10 different individuals. The results from these tastings were inconsistent. Apparently no constant variations in flavor were present.

Summary

1. An outline of a procedure suitable for growing and analyzing crops to determine the effects upon quality that result from various treatments with boron and manganese is presented.

2. Tomatoes showing manganese and boron deficiencies were grown during this investigation. Beets with marked evidences of boron deficiency were also obtained. Descriptions of these deficiencies as affecting foliage and fruit of the tomato and foliage and roots of the beet are given.

3. Results of analyses of plant samples from deficient and control plants are reported.

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THE B VITAMIN CONTENT OF BUDS AND SHOOTS OF SOME COMMON TREES[1]

PAUL R. BURKHOLDER AND ILDA MCVEIGH

(WITH TWO FIGURES)

Quiescent plant organs such as seeds, tubers, buds, etc., are among the most favorable materials for the study of growth because they are available in quantity, can be stored readily, and can be induced to grow easily by appropriate, simple manipulations of the environment. Numerous investigations of the processes and constituents of mature and sprouting seeds have resulted in a large body of descriptive data which at best may be said to represent a mere beginning in the complete story of plant growth. The metabolic transformations of carbohydrate, fat, and protein in growing seedlings are regulated by enzyme systems, and from studies on these regulating substances significant facts and principles are emerging. From the general field of metabolism we know that such vitamins as thiamine, riboflavin, and nicotinic acid play very important rôles in specific processes associated with energesis. Our studies on the vitamins of mature and sprouting seeds have shown significant increases in the content of several vitamins, including riboflavin and niacin, during germination (**2**). Little is known concerning the mechanism by which the amounts of these vitamins, and presumably also vitazymes, are augmented in plant tissues undergoing renewed growth activity; still, from the general field of comparative biochemistry, it seems reasonable to conclude that this class of substances has profound significance in the physiology of growing seedlings.

Other quiescent plant materials worthy of consideration from the standpoint of their chemical regulators are the winter buds of trees and shrubs in temperate latitudes. The expanding buds and shoots of trees have been shown to contain substances which stimulate the growth of yeasts (**4**, **5**). In the spring, auxin in woody plants appears to be generated first in the swelling buds and from there probably moves basipetally and stimulates cambial activity in the branches (**1**). A report on the thiamine found in buds, leaves, and bark of some common trees has been presented earlier (**3**). In the present paper results of microbiological assays for eight B vitamins in the buds, leaves, or shoots of 18 kinds of woody plants are reported. It is hoped that these data may have some value to students of tree growth and to those who are concerned with the conservation of wild life.

Materials and methods

Materials of the species listed in table I were collected periodically during the spring of 1944 from trees and shrubs growing in New Haven, Connecticut. Buds and young leafy shoots resulting from growth in the current

[1] This work was supported in part by the Nutrition Foundation, Inc., New York City.

TABLE I

VITAMIN CONTENT AND DRY MATTER OF SOME CONIFER NEEDLES AND BUDS OF DECIDUOUS TREES SAMPLED FEBRUARY 28, 1944. VALUES ARE EXPRESSED AS MICROGRAMS PER GRAM OF DRY MATTER

MATERIAL	THIAMINE	RIBOFLAVIN	PYRIDOXINE	NIACIN	INOSITOL	BIOTIN	PANTOTHEN	VITAMIN B_c	DRY MATTER
	γ	γ	γ	γ	γ	γ	γ	γ	%
Tsuga canadensis, hemlock (needles)	7.0	10.6	25.2	44.2	2220	0.169	6.5	3.31	58.0
Pinus strobus, white pine (needles)	4.6	7.6	33.7	25.5	1536	0.006	17.7	2.13	45.9
Quercus alba, white oak	3.0	3.0	3.8	9.6	375	0.097	6.2	0.25	37.4
Betula lenta, black birch	3.7	3.3	12.8	19.4	723	0.374	10.2	0.18	66.3
Fagus sylvatica, beech	4.6	2.7	14.6	15.5	900	0.355	10.3	0.10	65.9
Acer rubrum, red maple	4.4	4.2	15.5	162.2	412	0.336	2.5	0.22	56.4
Pyrus malus, apple	3.9	4.6	26.0	14.6	437	1.380	10.0	0.31	54.4
Aesculus hippocastanum, horse chestnut	3.4	2.1	13.3	14.4	1118	0.518	4.2	0.13	49.1
Acer platanoides, Norway maple	3.1	.....	13.1	16.8	384	0.071	.....	0.12	55.8
Liriodendron tulipifera, tulip-tree	10.8	4.5	35.9	12.0	1118	0.193	9.5	1.01	54.1
Ulmus americana, elm	3.3	3.3	13.5	12.7	1781	0.221	6.5	0.62	61.1
Forsythia intermedia, Forsythia	12.6	8.4	24.2	26.8	2506	0.113	9.2	1.47	61.0
Alnus rugosa, alder	4.2	2.8	20.3	23.3	636	0.764	7.1	0.09	59.6
Sassafras officinalis, sassafras	4.7	4.9	20.8	15.4	460	0.433	15.9	0.69	73.8
Populus grandidentata, poplar	6.4	5.0	49.3	19.1	1340	0.379	4.7	0.68	49.8
Salix discolor, pussy willow	7.7	5.0	28.7	20.8	2040	1.600	6.1	0.45	53.0
Tilia americana, linden	3.8	3.3	12.7	13.5	523	0.605	2.6	0.16	53.4
Platanus occidentalis, sycamore	4.2	.....	14.3	13.1	646	0.405	6.4	0.08	55.6

season were harvested, weighed, and dehydrated rapidly under reduced pressure in a lyophilizer. Materials sampled on May 29, when the leaves had attained approximately mature size, included only leaves. The dried materials were weighed and stored in a desiccator in darkness until assays could be performed on all of the samples. In preparation for the assays, the dried materials were ground in a small Wiley mill, and aliquots of 500 mg. were placed in acetate buffer solution at pH 4.5 for the purpose of obtaining extracts. Digestion of the samples with enzymes and the specific assay procedures for each of the B vitamins were carried out in the same manner as reported in a preceding paper (**2**).

Results

The data obtained in assays for 8 B vitamins in quiescent buds of 16 species of deciduous trees and shrubs and in the leaves of 2 conifers harvested

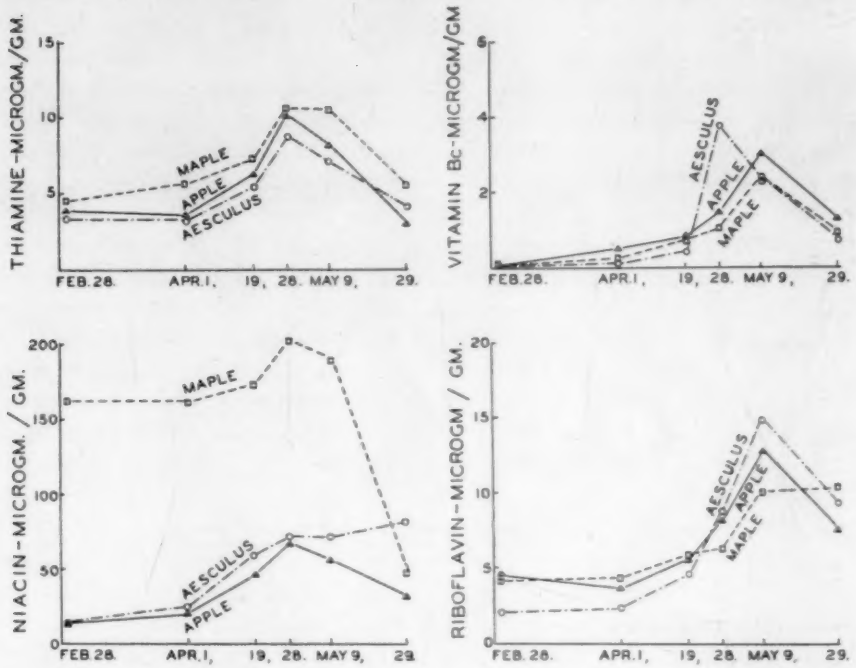

FIG. 1. Vitamins in buds and shoots of *Aesculus hippocastanum* (horse chestnut), *Pyrus malus* (Baldwin apple), and *Acer rubrum* (red maple), sampled at intervals in the spring of 1944.

February 28, 1944, are presented in table I. All values have been expressed as micrograms of vitamin per gram of dry matter. Notably high in thiamine are the leaves of hemlock and the winter buds of tulip, willow, and Forsythia. The needles of both hemlock and pine appear to have more riboflavin than the buds of the deciduous trees, although the buds of Forsythia contain relatively high concentrations of this vitamin. Pyridoxine appears

TABLE II

VITAMIN CONTENT AND DRY MATTER OF BUDS AND SHOOTS OF DECIDUOUS TREES SAMPLED AT SUCCESSIVE INTERVALS IN THE SPRING OF 1944. VALUES ARE EXPRESSED AS MICROGRAMS PER GRAM OF DRY MATTER

MATERIAL	DATE OF SAMPLING	THIAMINE	RIBOFLAVIN	PYRIDOXINE	PANTOTHEN	INOSITOL	BIOTIN	NIACIN	VITAMIN B_c	DRY MATTER
		γ	γ	γ	γ	γ	γ	γ	γ	%
Horse chestnut	Feb. 28	3.4	2.1	13.3	4.2	1118	0.52	14.4	0.13	49.1
	Apr. 1	3.2	2.3	8.2	2.9	1542	0.52	23.7	0.05	47.2
	Apr. 19	5.5	4.7	13.0	3.5	2521	0.95	59.0	0.45	32.8
	Apr. 28	8.8	8.7	9.1	6.6	5364	2.26	70.8	3.79	21.0
	May 9	7.1	15.0	9.2	5.1	6500	1.52	71.1	2.57	22.4
	May 29	4.0	9.4	.....	.....	.....	.....	83.7	0.81	29.2
Baldwin apple	Feb. 28	3.9	4.6	.....	10.0	437	1.38	14.6	0.31	54.4
	Apr. 1	3.5	3.7	2.6	7.9	756	1.19	21.4	0.51	48.0
	Apr. 19	6.3	5.7	1.7	22.0	715	1.20	46.0	0.86	34.8
	Apr. 28	10.3	8.3	5.9	26.1	1874	1.29	69.6	1.48	26.4
	May 9	8.4	12.9	5.9	12.4	2167	1.57	56.2	3.16	27.2
	May 29	2.8	7.7	.....	.....	.....	.....	31.6	1.35	34.7
Black birch	Feb. 28	3.7	3.3	12.8	10.2	723	0.37	19.4	0.18	66.3
	Apr. 1	3.1	2.8	9.2	13.1	640	0.44	25.1	0.15	58.8
	Apr. 19	2.1	3.6	8.9	11.8	820	0.42	26.3	0.07	53.2
	Apr. 28	3.2	4.0	6.2	14.3	2600	0.49	29.9	0.21	42.9
	May 9	6.6	15.8	5.6	10.1	8686	0.65	63.0	2.54	29.3
	May 29	5.7	11.9	.....	.....	.....	.....	33.6	1.12	42.4
American elm	Feb. 28	3.3	3.3	13.5	6.5	1781	0.22	12.7	0.62	61.1
	Apr. 1	3.2	2.3	5.1	4.8	1912	0.38	17.9	0.68	49.7
	Apr. 19	3.6	4.3	4.5	12.6	1830	0.33	44.7	0.83	30.5
	Apr. 28	4.9	5.4	4.4	15.0	2308	0.35	45.5	1.10	24.1
	May 9	9.7	11.7	11.2	15.2	10140	1.22	64.0	3.77	23.7
	May 29	4.7	8.7	.....	.....	.....	.....	31.1	2.14	31.7
Red maple	Feb. 28	4.4	4.2	15.5	2.5	412	0.34	162.2	0.22	56.4
	Apr. 1	5.7	4.3	15.0	2.4	634	0.65	160.9	0.24	43.3
	Apr. 19	7.4	6.0	19.3	4.1	2557	0.67	172.5	0.78	35.3
	Apr. 28	10.6	6.3	18.5	5.9	4188	0.63	202.0	1.13	29.5
	May 9	10.5	10.0	19.6	7.6	3586	0.81	189.5	2.57	26.5
	May 29	5.7	10.9	.....	.....	.....	.....	48.2	0.94	28.7

to be relatively abundant in buds of poplar, Forsythia, tulip, apple, and in the coniferous needles. Niacin is high in alder, willow, poplar, birch, Forsythia, and conifer needles, and very high in the buds of red maple. Inositol is high in the conifer needles and in buds of elm, Forsythia, and willow. Biotin is low in the conifer needles and high in apple, horse chestnut, alder, sassafras, linden, and willow. Pantothenic acid is relatively high in pine needles and buds of birch, beech, and sassafras. Vitamin B_c is high in hemlock and pine needles and in buds of tulip and Forsythia.

Buds from five kinds of trees, Baldwin apple, horse chestnut, black birch, American elm, and red maple were sampled on the following dates: Feb. 28, Apr. 1, Apr. 19, Apr. 28, May 9, and May 29, 1944. The data obtained in the vitamin assays and the water content expressed as percentage of the

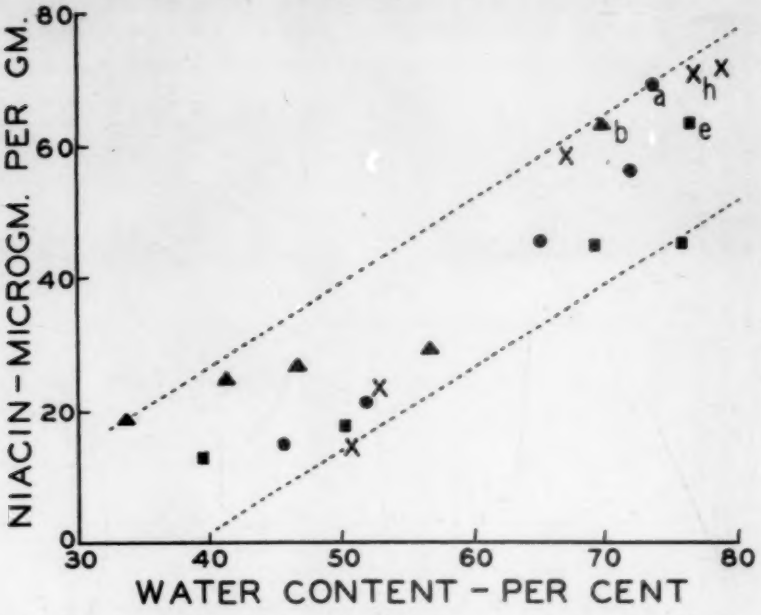

FIG. 2. Increase of niacin during the period of increased hydration of the tissues in expanding buds of apple (a), horse chestnut (h), birch (b), and elm (e).

fresh weight are presented in table II. Data obtained for thiamine, niacin, B_c, and riboflavin in maple, apple, and horse chestnut are presented graphically in figure 1. The graphs show that these vitamins increase in concentration, expressed on a dry weight basis, during the period of bud expansion and shoot development. Toward the end of May, when the leaves had attained approximately their full size, obvious decreases were found in the vitamin concentration, and the percentage of dry matter was observed to increase.

The increase of niacin along with the gain in water content of the expanding buds of four kinds of deciduous trees, sampled Feb. 28, April 1, 19, 28, and May 9, is presented in figure 2. Niacin concentration, plotted as micro-

grams per gram of dry matter, shows a rough direct relationship to the water content plotted as percentage of the fresh bud material. A similar increase of niacin in seedlings of *Avena sativa* during the period of rapid growth and gain in water content has been pointed out in a previous report (**6**). Perhaps the increase of niacin is related to specific hydrolytic processes which result in the liberation of bound precursor materials, which are then converted to active vitamin in the growing tissues. The situation appears to be complicated by the possibility that mobilization of vitamin materials from the rest of the plant into the shoot apices may occur to an appreciable extent. Studies on vitamin production in germinating seeds yield more convincing data with respect to the production of these compounds because the whole plant or any of its principal parts can be analyzed directly. A simplified approach to the problem of vitamin synthesis in higher plants might be found in perfusion or controlled feeding experiments with isolated organs. Investigations of this type have already been performed with root cultures (**7**).

Earlier studies on the production and distribution of auxin in shoots of woody plants led to the conclusion that the buds and new shoots which develop from them are centers of hormone synthesis (**1**). Movement of hormone from the bursting buds basipetally into older portions of the stem was followed by renewed cambial activity progressively farther back from the tips of the branches. The authors (**1**) concluded that "it is highly probable that growth hormone acts as a cambial stimulator, but it is not yet possible to suggest the extent to which any specific substance may be involved." The increases in concentration of thiamine, riboflavin, and niacin, which are important specific substances concerned in intermediate metabolism, suggest possible important rôles of some regulating substances in addition to auxin which may be concerned with renewed growth activity of woody plants in the spring.

The significance of these data for wild life lies in the fact that B vitamins are present in the winter buds and in increased amounts in the leafy shoots of deciduous woody plants, and in the needles of conifers. Browsing animals and birds which may feed on buds and shoots can obtain their essential vitamins from these plant materials.

Summary

Microbiological assays are reported for thiamine, riboflavin, pyridoxine, niacin, inositol, biotin, pantothenic acid, and vitamin B_c occurring in the buds and leaves or shoots of 18 kinds of woody plants. The possible significance of the data for studies on growth and for problems in the conservation of wild life is discussed briefly.

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EFFECT OF COPPER AND ZINC DEFICIENCIES ON THE PHOTOSYNTHETIC ACTIVITY OF THE FOLIAGE OF YOUNG TUNG TREES

ARNAUD J. LOUSTALOT, FRED W. BURROWS, SEYMOUR G. GILBERT, AND ALVIN NASON[1]

Deficiencies of the minor elements have been recognized as limiting factors in the profitable production of tung oil from almost the very start of the industry. In recent years disorders of tung foliage caused by deficiencies of zinc, manganese, copper, iron, and magnesium have been reported (**1, 2, 3, 10, 11**). A deficiency of one or more of these elements may so upset the normal functions of the plant as to result in symptoms of a pathological nature. For example, a deficiency of zinc causes deformation, bronzing, crinkling, and one-sided development of the leaves. The severity of the diseased condition is not uniform throughout the tree. Some of the shoots are stunted, which results in unbalanced growth of the tree. Copper deficiency causes a characteristic cupping of the terminal leaves which are usually abnormally small and have interveinal chlorosis. These abnormalities are evidence that an insufficient amount of either of the above elements affects the metabolic processes, yet relatively little work has been published as to the effect of such deficiencies on the physiological processes involved. In 1942 REUTHER and BURROWS (**12**) reported that the photosynthetic activity of manganese-deficient tung foliage was not greatly impaired as a result of the deficiency. They point out, however, that manganese-deficient trees tend to have small leaves and thus the total photosynthate produced would be less than that of normal trees even though the efficiency of the foliage per unit area was not greatly affected.

In October, 1942, preliminary work was started at this station to determine the effect of copper deficiency on the photosynthetic activity of tung foliage. Biochemical studies (**4**) had shown that the carbohydrate metabolism of copper-deficient leaves was seriously impaired, as was evidenced by their low starch content in comparison with normal leaves. The results of the preliminary study of photosynthesis indicated that copper deficiency reduces the rate of photosynthesis, but the data obtained were incomplete because of the lateness of the season and interruption of the work due to the entrance of two of the authors into the army. It was therefore considered advisable to obtain further information and in 1943 a more comprehensive experiment was planned to determine the effects of both copper and zinc deficiency on the photosynthetic activity of tung foliage. The results of the experiments in both 1942 and 1943 are herein reported.

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Materials and method

The apparatus and procedure used to determine the rate of apparent photosynthesis were similar to those described by HEINICKE and HOFFMAN (**5**). The HEINICKE cup (**6**) was used as an assimilation chamber and was attached to the lower surface of individual tung leaves. Air was drawn through the cups by mercury pumps at the rate of 2 to 2½ liters per 100 square centimeters per hour. In 1942 the total volume of air passing through the absorption unit was estimated by calculation from the average of three flow measurements made during the course of a run by means of a wet-test flow meter; in 1943 the total volume of air was determined directly by individual wet-test flow meters connected to each absorption tower. In 1942 a single unit, having six absorption towers, was used; two of the towers were used as air checks and four were available for the experimental leaves. In 1943 two units, each with six absorption towers, were available. Thus it was possible to use two towers as air checks and one each for ten experimental determinations.

The material for the study in 1942 consisted of one-year-old seedling trees growing on a Fort Mead loamy fine sand near Alachua, Florida. Three types of trees were selected as follows: (1) trees apparently normal, showing no evidence of a deficiency; (2) deficient trees with the characteristic symptoms of copper deficiency; and (3) copper-treated trees. These latter trees had shown deficiency symptoms prior to treatment on July 8, 1942, when 5 grams of copper sulphate in solution were applied to the soil at the base of each tree. By August 5 the treated trees had apparently made complete recovery (**3**). Two replications of four trees each were used. Each replication consisted of one treated, one normal, and two copper-deficient trees. The copper content, in p.p.m., in the leaves of the three types of trees was as follows: normal, 3.5; deficient, 3.1; and treated, 4.0. The rate of apparent photosynthesis was determined daily from October 6 through October 9. Two determinations were made each day; one in the morning and one in the afternoon. The six carbon dioxide absorption towers were utilized in the following manner: One was connected to a leaf on a normal tree; one to a leaf on a tree that had recovered after treatment; two were connected to individual leaves each on a separate deficient tree and the remaining two towers served as air checks. Determinations of apparent photosynthesis were made for two consecutive days on each replication.

The trees used in the 1943 experiments were two-year-old seedlings growing on the Minor farm of the University of Florida Agricultural Experiment Station near Gainesville. The trees were planted eight feet apart, on the square, in an area that is deficient in both copper and zinc, and had been treated in 1942 with either copper or zinc or both. In the late summer of 1943 trees that had been treated in 1942 with both copper and zinc showed no deficiency symptoms; those treated with only copper showed zinc-deficiency symptoms; and those treated with only zinc showed copper-deficiency symptoms. The experiment was laid out with three replications, each con-

sisting of a normal, a copper-deficient, and a zinc-deficient tree. About three weeks before the experiment was started all the trees received 12 liters of HOAGLAND and ARNON's full nutrient solution (**8**) from which copper and zinc were omitted. The normal trees in addition each received a solution containing 10 gm. of copper sulphate and 10 gm. of zinc sulphate in a liter of water; the zinc-deficient trees each received a solution containing 10 gm. of copper sulphate; and the copper-deficient trees each received a solution containing 10 gm. of zinc sulphate. The solutions were applied in all instances in a circle about 70 cm. in diameter on the soil at the base of the tree. No chemical tests were made to determine the extent to which these nutrients were absorbed by the tree but throughout the course of the experiment the check trees continued normal and the deficient trees continued to show only the respective symptoms exhibited in the beginning.

In 1943 determinations of the rate of apparent photosynthesis began on September 17 and continued until November 10, when frost destroyed the leaves. On the trees showing either copper or zinc deficiency, a comparison of the photosynthesis rate was made of normal-appearing leaves with those showing definite deficiency symptoms but without necrosis. Two leaves of each type were used on each copper- or zinc-deficient tree. Two leaves in comparable positions were used on each normal tree. Whenever feasible terminal leaves were used, otherwise leaves as near the terminal as possible. Since the number of carbon dioxide absorption towers available equaled the number of experimental leaves of the trees of one replication, it was necessary to determine the rate of apparent photosynthesis for the three replications in rotation, the determinations being run during two days in each instance. Thus during each week two days' readings were obtained from each replication. As a rule two determinations were made daily, one in the morning from 9:00 A.M. to 12:00 M. and one in the afternoon from 12:15 P.M. to 4:15 P.M. The same leaves were used for the two successive days' determinations for one replication in any given week. During the succeeding week, a different set of leaves on each tree was used.

Results and conclusions

The data obtained in 1942 show that the apparent photosynthetic activity of copper-deficient leaves was considerably lower than that of leaves on either normal or treated trees. As an average of 7 determinations, the milligrams CO_2 assimilated per 100 square centimeters per hour were 14.01 for leaves of copper-treated trees, 12.25 for normal trees, and 2.92 and 5.78, respectively, for the two copper-deficient trees. Statistical analysis of these data indicates that the difference between the rate for leaves on normal plants and that for leaves on treated plants is not significant, but that the difference in rate between these two and the deficient leaves is significant at the 1 per cent. level.

The data obtained in 1943, and given in table I, show that both copper and zinc deficiency considerably reduced the rate of apparent photosynthesis

TABLE I

MEAN RATE OF APPARENT PHOTOSYNTHESIS OF TUNG TREES AS AFFECTED BY DEFICIENCY OF COPPER AND OF ZINC; BY SEASON AND BY TIME OF DAY

DEFICIENCIES			SEASON		TIME OF DAY	
PLANT	LEAF	CO_2 ABSORBED*	PERIOD	CO_2 ABSORBED†	AVERAGE ALL READINGS	CO_2 ABSORBED‡
		mg./100 cm.²/hr.		*mg./100 cm.²/hr.*		*mg./100 cm.²/hr.*
Normal	Normal	11.58	9/17–23	4.56	Morning	6.60
			9/24–30	6.08		
			10/8–14	5.82	Afternon	6.03
Copper-deficient	Normal	5.20	10/15–23	8.38		
	Abnormal	2.19	10/21–30	6.11		
Zinc-deficient	Normal	8.15				
	Abnormal	4.45				
L. D. Sig. @ 0.05		1.52		0.81		0.43
L. D. Sig. @ 0.01		2.20		1.07		0.57
L. D. Sig. @ 0.001		3.31		1.38		0.74

* Each entry is the mean of 54 readings.

† Each entry includes readings made on normal leaves, copper-deficient leaves, and zinc-deficient leaves. All entries are means of 60 readings except entry for the period 10/21–30, which is the mean of 30 readings. For comparing the last period with any one of the first four, least difference significant at 0.05 is 0.99; at 0.01, 1.31; and at 0.001, 1.69.

‡ Each entry is the mean of 135 readings and includes the data for determinations made on normal leaves, copper-deficient leaves, and zinc-deficient leaves.

in leaves of young tung seedlings. Although the reduction was the greatest in the case of leaves that showed the deficiency symptoms there was a very highly significant reduction in the rate of carbon dioxide assimilation even for those leaves that appeared normal but were on deficient plants. This would indicate that, in the case of copper deficiency especially, the decrease in apparent photosynthesis observed is not primarily associated with the appearance of chlorosis or necrosis of the leaf tissue. The average rate of carbon dioxide assimilation for leaves on normal trees was 11.58 milligrams per 100 square centimeters per hour as compared with 8.15 and 5.20 milligrams, respectively, for normal-appearing leaves on zinc- and on copper-deficient trees. The average rates for abnormal leaves on zinc- and on copper-deficient plants were 4.45 and 2.19 milligrams, respectively. Statistical analysis shows that all differences are significant at the 0.001 or 0.01 levels.

It is likewise demonstrated that zinc deficiency to the degree in which it occurred in this experiment reduced the rate of apparent photosynthesis much less than did copper deficiency as it occurred on the trees used, the difference being supported by very high statistical odds. Judging by the appearance of the trees, the deficiencies of copper and zinc were equally severe, since about the same proportion of total foliage was affected in each case.

The data also indicate that with these young trees, the rate of photosynthesis was greatest about the middle of October. This is probably due to the fact that more favorable climatic conditions prevailed during this period than in the previous weeks when the weather was cloudy and there was considerable rainfall.

That the rate of photosynthesis was somewhat lower in the afternoon than in the morning, the difference being statistically significant at the 0.01 level, is also shown by the data. This is in agreement with work on apples and pecans. HEINICKE and CHILDERS (**7**) found in the case of the apple that the rate of CO_2 assimilation was greatest in the interval between 9:30 A.M. and 2:30 P.M. Likewise in the case of the pecan (**9**) higher rates of photosynthesis were observed in the forenoon than in the afternoon. The accumulation of the products of photosynthesis, partial or complete closing of the stomates, and the higher air temperature which normally occurred in the afternoon, are some of the factors that would tend to reduce the amount of apparent photosynthesis in the afternoons.

An analysis of variance indicates that there are no statistically significant interactions between time of day and season, between treatment and time of day, or between treatment and season.

Summary

Copper and zinc deficiencies were definitely associated with a reduced rate of apparent photosynthesis in leaves of young tung trees. The amount of reduction was less in the case of zinc deficiency than for copper deficiency

as they occurred in this experiment. There was a highly significant reduction in carbon dioxide assimilation even with normal-appearing leaves on deficient plants. The highest rates of photosynthesis in the fall occurred about the middle of October and were associated with favorable weather conditions. As a rule the rates were higher in the mornings than in the afternoons.

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A GERMINATION INHIBITOR IN THE SEED COATS OF CERTAIN VARIETIES OF CABBAGE

L. G. Cox, H. M. Munger, and E. A. Smith

Introduction

Freshly harvested seed of #1339–1–5, a selection made by the late C. H. Myers, from Wisconsin Hollander, and various strains of Glory cabbage exhibit a pronounced dormancy and uneven germination, thus extending the breeding cycle from 1 to 2 years. This type of dormancy gradually disappears during several weeks of air-dry storage at room temperature, but by this time it is too late for field planting of seed produced in the greenhouse.

We have been unable to find references in the literature to any type of seed dormancy in *Brassica oleracea*, but various types of more or less temporary dormancy in other species of Brassica seed have been reported from time to time. Kidd and West (**1**) were able to induce secondary dormancy in the seed of *Brassica alba* by low partial pressures of carbon dioxide. Later Kidd and West (**2**) found that the seed coats of *Brassica alba* restricted the gas exchange of the embryo during the early stages of seed maturity, but following maturity the seed coats gradually lost their ability to restrict gas exchange, along with the loss of vital activity of their cells. Takiguti (**5**) reported that immature rape seed (*Brassica napella*) germinated readily, but when the embryo reached full development, germination of both intact seed and excised embryos dropped significantly. By the time the seed coat had completely matured the temporary dormancy of the embryo had disappeared, but the seed coats still restricted germination. Toole and Toole (**6**) tested samples of seed of *Brassica alba*, *B. juncea*, and *B. campestris* for germination at various lengths of time following harvest and found that the rate of germination increased with the length of the storage period.

A growth-inhibiting substance in new crop mustard seeds has been reported by Shuck (**4**).

Results

Effect of seed coats on germination of freshly harvested cabbage seed

Pods containing mature seed were collected on April 26, 1944, and stored at 4° C. until April 30.

The fresh seeds were sterilized for 15 minutes in calcium hypochlorite (**7**). As a control, 50 intact seeds were placed on Randolph and Cox's nutrient agar (**3**) in 1-ounce screw-cap bottles. The seed coats of a second lot of 50 seeds were slit longitudinally so as to expose the radicle and cotyledons, thereby removing any restrictions to gas exchange. The seed coats were removed entirely from a third lot of 39 seeds and the embryos placed directly on agar. All cultures were placed in the dark at 30° C. Geotropic curva-

TABLE I

EFFECT OF SEED COATS ON GERMINATION OF FRESHLY HARVESTED CABBAGE SEED

TREATMENT	NUMBER OF SEEDS	PERCENTAGE GERMINATION		
		0 DAYS	1 DAY	2 DAYS
		%	%	%
Control	50	0	20	60
Seed coat slit	50	0	16	60
Seed coat removed	39	0	84	84

ture of the radicle was taken as an index of germination. The results are summarized in table I.

The practically identical germination of intact seed and seed with exposed radicles and cotyledons suggests strongly that the delay in germination is not due to the restriction of gas exchange. The immediate germination of excised embryos suggests that some inhibitor in the seed coats is responsible for the delayed germination of intact seed.

The average rate of growth of the radicle following germination was greatest for the seedlings from which the seed coats had been removed.

EFFECT OF SEED COATS ON THE GERMINATION OF RECENTLY HARVESTED AIR-DRIED CABBAGE SEED

Seeds were removed from the pods and carefully air dried at room temperature for a few days. An experiment similar to Experiment 1 was repeated with this lot of seed. The results are summarized in table II.

When the percentages of germination of freshly harvested seed (table I) are compared at the end of 2 days with those of recently harvested air-dried seed (table II), it is seen that the rate of germination of the excised embryos and of seed with slit seed coats is unaffected by drying the seed coat. The rate of germination of intact seed, on the other hand, has been significantly decreased by the drying process. Drying of the seed coats, therefore, appears to induce certain changes in the seed coats which restrict gas exchange, but do not appear to affect the inhibitors in the seed coats.

Restriction of the entry of water by the seed coats, as a possible limiting factor, is eliminated by the fact that air-dried seeds imbibe their maximum moisture within a few hours after soaking.

TABLE II

EFFECT OF SEED COATS ON GERMINATION OF RECENTLY HARVESTED AIR-DRIED CABBAGE SEED

TREATMENT	NUMBER OF SEEDS	PERCENTAGE GERMINATION			
		0 DAYS	1 DAY	2 DAYS	3 DAYS
		%	%	%	%
Control	50	0	4	28	48
Seed coat slit	45	0	7	53	66
Seed coat removed	50	0	72	84	86

THE EFFECT OF EROSION OF THE SEED COAT BY CONCENTRATED SULPHURIC ACID ON THE RATE OF GERMINATION OF RECENTLY HARVESTED AIR-DRIED SEED

Groups of 50 seeds from the same lot as used in the previous experiment, were tested for various periods of time in cold concentrated H_2SO_4 (4° C.), soaked in an excess of 250 cc. of cold tap water for 25 minutes, and placed in Petri dishes on 5 layers of moist filter paper in the dark at 30° C. The results are summarized in table III.

Erosion of the seed coats by cold concentrated sulphuric acid for periods up to 8 minutes gave increased rates of germination over the controls. The optimum treatment at the end of 107 hours lay between 1 and 4 minutes;

TABLE III

EFFECT OF EROSION BY SULPHURIC ACID ON THE GERMINATION OF DORMANT CABBAGE SEED

TREATMENT WITH H_2SO_4	PERCENTAGE GERMINATION					
	0 HRS.	11 HRS.	21 HRS.	35 HRS.	62 HRS.	107 HRS.
min.	%	%	%	%	%	%
0	0	0	0	10	32	54
1	0	0	52	78	78	88
2	0	4	66	72	72	76
4	0	16	38	62	62	86
8	0	10	14	38	38	68
15	0	4	4	4	4	4
30	0	0	0	0	0	0

i.e., 2 minutes soaking appears to be a safe period of treatment. Cold concentrated sulphuric acid is preferred to that at room temperature, since it prevents the heat generated by the corrosion of the seed coat from reaching a level injurious to the embryo.

The percentage germination of seed treated for 1- or 2-minute periods was 78 and 72, respectively, at the end of 35 hours. This is considerably higher than the 53 percentage germination of seeds with slit coats at the end of 48 hours (table II). Erosion by sulphuric acid probably killed many of the cells of the seed coat containing the germination inhibitor, and the subsequent leaching in tap water probably reduced markedly the inhibiting substance or substances.

THE EFFECT OF THE WATER-SOLUBLE FRACTION OF ALCOHOLIC EXTRACTS OF SEED COATS ON GERMINATION OF PARTIALLY DORMANT INTACT SEED

The seed coats were removed from partially dormant cabbage seed, ground to a fine powder with fine quartz sand in a mortar, and the powder soaked in a small amount of 95 per cent. alcohol for 24 hours. The alcoholic extract was decanted, centrifuged in an angle-head centrifuge to remove sand and cellular debris, and then the alcohol evaporated at 70° C. A small

TABLE IV

EFFECT OF WATER-SOLUBLE ALCOHOLIC EXTRACTS OF SEED COATS ON GERMINATION OF PARTIALLY DORMANT CABBAGE SEED

TREATMENT	PERCENTAGE GERMINATION				
	0 DAYS	2 DAYS	3 DAYS	5 DAYS	12 DAYS
	%	%	%	%	%
Control	0	22	80	82	82
Seed-coat extract	0	8	52	72	94

amount of distilled water was added to the extract and the mixture allowed to reach equilibrium for 24 hours at 4° C. The water extract was then used to moisten 50 dry seeds of the same lot of partially dormant seeds placed on 5 sheets of filter paper in a Petri dish. A second lot of 50 seeds serving as a control was watered with distilled water and then both lots were placed at 30° C. in the dark to germinate. The results are summarized in table IV.

The seed-coat extract definitely inhibited germination for at least 5 days. The increase in germination percentage of extract-treated seed over that of the controls at the end of 12 days is difficult to explain, but it does at least indicate that the seed-coat extracts are non-toxic.

Practical applications

The practical application of the results of the preceding experiments was achieved by soaking the seed in cold concentrated sulphuric acid (4° C.) for 1 minute and draining on a screen for 1 minute. The seeds were then washed in cold running tap water and then either dried on blotting paper overnight or allowed to soak in an excess of tap water overnight and then allowed to dry on blotting paper for a short time before planting.

An experiment (table V) was designed to investigate the effect of sulphuric acid treatment, immediate drying following treatment, soaking overnight, and the effect of mercuric bichloride (1:4000) which has customarily been sprinkled on the seed flats to prevent damping off. The seeds were sown in flats on a mixture of sand, peat moss, and soil, and germinated in the greenhouse. Seed from C. H. MYER's selection #1339–1–5, harvested dur-

TABLE V

EFFECT OF SULPHURIC ACID TREATMENT, SOAKING AND MERCURIC CHLORIDE ON GERMINATION

BEFORE PLANTING	AFTER PLANTING	PERCENTAGE GERMINATION ON 5/23/44
		%
No treatment	$HgCl_2$	31
2 min. conc. H_2SO_4 not soaked	$HgCl_2$	57
No treatment	No $HgCl_2$	27
2 min. conc. H_2SO_4 soaked	No $HgCl_2$	71
2 min. conc. H_2SO_4 not soaked	No $HgCl_2$	48

ing April and planted in early May, gave only about 2 per cent. prompt germination. The following test was run on lot 18–4 of the above selection. Treatments were made on May 15.

The results indicate that soaking of the acid-treated seed definitely increased the germination percentage over that for unsoaked seed. This could be caused either by the removal of traces of sulphuric acid or by inhibiting substances present in the seed coat which were released by the acid treatment. Mercuric bichloride has no significant effect on either intact or acid-treated seed.

Summary

Freshly harvested seed of selection #1339–1–5 made by C. H. MYERS, from Wisconsin Hollander, and strains of Glory cabbage exhibit a pronounced dormancy and uneven germination, thus requiring 2 years for the breeding cycle. Dormancy gradually disappears during several weeks of air-dry storage at room temperature.

By means of embryo culture techniques in which intact seed, seed with the seed coats split to allow gas exchange, and completely excised embryos, it was shown that the fresh seed coats inhibited germination other than by restricting gas exchange. Seed coats of freshly harvested air-dried seed, however, may partially restrict gas exchange.

The water-soluble fraction of alcoholic extracts from the seed coats of partially dormant seed, when applied to seed of the same lot retarded germination, thus indicating the presence of a germination inhibitor.

A practical method of breaking this dormant condition consists of soaking the dry seed for 1 minute in cold concentrated sulphuric acid (4° C.), allowing the excess acid to drain for 1 minute, washing thoroughly in cold running tap water, and then soaking in an excess of tap water for several hours prior to planting.

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A SIMPLE AND INEXPENSIVE VOLTAGE REGULATOR FOR LABORATORY USE

R. H. WALLACE

(WITH FOUR FIGURES)

Variations in voltage are frequently a source of trouble in experimental work resulting in unreliable data, injury to equipment, or shortened life of electrical devices. Considerable effort has been expended in attempts to solve this fundamental problem but no wholly successful regulator for all purposes has yet been developed due to the diversity of voltage requirements. Some devices require essentially instantaneous correction whereas others can tolerate appreciable lag, but must have no distortion of wave form.

There are in general three types of voltage controls. Perhaps the best known and most commonly used of these is the electromagnetic type (no moving parts) in which a change in the flux of the field or some similar system is used so that the output voltage remains constant for great differences of input. This type has the definite advantage of being very rapid, usually correcting within one cycle, but has the disadvantage that it distorts the wave form. For this reason motors and other equipment affected by wave form and power factor function poorly or not at all. In addition, in order to get sufficient amperage supplying power, a relatively small (10 amp.) model must weigh about 75 lbs. For larger sizes, the weight is proportional. In some devices, such as photolometers, even these controls cause "flares" in the bulbs used and hence drifts of null.

A second general type of voltage regulator is that obtained with a complicated circuit of radio tubes. They have voltage control in the general range of hundredths of a volt for outputs of 110 volts and are highly desirable for certain specific uses. They are very limited in application, however, and will not be described further.

A third type of regulator is the fundamental one that has been used in the electrical industry since its inception. It consists essentially of a transformer with exposed windings so that a movable contact can pass across them and thus pick up any voltage desired. This general form is broadly used on rural power lines. They are automatically operated and are set to hold the voltage within certain limits. This type of control can be built with any capacity and almost any tolerance. It has the very great advantage of giving no wave distortions and of having a high efficiency. It has the very distinct disadvantage of time lag, for it takes from one to several seconds to correct line variations. Recently, in working with a recording potentiometer, voltage variations from 90 to 120 were encountered. An electromagnetic regulator of the sola type was available but the recorder would not function when this was used as voltage supply. The potentiometer has a small induction motor and several radio tubes. Thus it was im-

possible to determine definitely which part or parts failed to function on this voltage source. The second type of voltage regulator was not available but it seemed from theoretical considerations that it could not work.

Since the third type of voltage regulator was not available in a small laboratory model, the present instrument was developed to supply this need. The present control is built as an autotransformer of the Variac or Variatron type. The latter seems to be better adapted to the scheme, primarily because of the wheel contact. Many laboratories already have these variable transformers and they can easily be converted to voltage controls.

This regulator is, in general, a midget model of the autotransformer type mentioned above as being used in power lines. It can be built to handle from a few to 50 amperes. It consists essentially of two circuits. The first of these is the autotransformer in which some windings are exposed. A contact traveling across these windings gives various output voltages. These transformers are available from several companies for many amperages. Figures 2, 3, and 4 show a Variatron model V-1 which is rated at 5 amperes at 110 volts. The second circuit is the control one and is so designed that line voltage variations unbalance it, causing a small motor to turn the adjusting shaft of the variable transformer, and add or subtract voltage by having the traveling contact pick up or drop out turns until the balance is restored.

Since voltage varies both above and below any set value, it is necessary that the motor (M) be reversible. This small induction motor was used in a previous work (**1**). It has a built-in gear reduction of 450 to 1 so that the torque delivered on the power shaft is more than adequate to move the contact back and forth across the turns. The motor has two starting transformers. When the leads to one transformer are shorted, current flows and the motor turns in one direction, while when the leads to the other transformer are shorted, the motor turns in the opposite direction. If both transformers are shorted simultaneously the motor stalls, but no injury or overheating results. It will be noted in figures 1 and 2 that the plates of two small thyratron tubes (2A4G) are placed in series in these transformer circuits to control the motor. The grid of one of these tubes is held plus by a small B-battery, while the grid of the other is held minus in the same manner. If this were all of the control circuit, the motor would turn continuously in the direction caused by the thyratron with the plus charge. The two set batteries (B and B_1) serve as a bucking circuit against which to play the variable circuit under control. A variable DC voltage is obtained from a 6H6 rectifier tube with a suitable transformer tied directly to the line at X. Three resistances are connected in series between the cathode and plate of this tube. The first of these (R_4) is 50,000 ohms and serves to give the correct voltage across R_2 and R_3. R_2 and R_3 are 3,000-ohm standard wirewound potentiometers with their variable arms connected to the plus of battery B and the minus B_1, respectively. The common junction between these resistances is connected back to the cathode of the two thyratrons, and over

to one side of the 110 AC. Since resistances R_2 and R_3 now show a pulsating DC voltage and are so connected that one becomes more plus and the other more minus as you proceed from their common junction, one can simply use this voltage to buck the two set batteries (B and B_1) and have a device responsive to line voltage variation.

As voltage at X decreases, one tube becomes more plus and fires, while the other becomes more minus and ceases to fire. When voltage varies in the other direction, the reverse firing action occurs. It is necessary to watch the phase of the voltage in the 6H6 tube, for the current both through it and

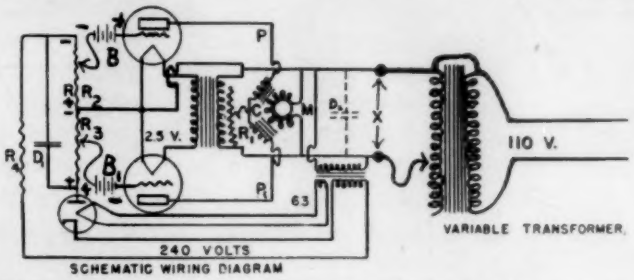


FIG. 1. Schematic diagram showing wiring of voltage control.

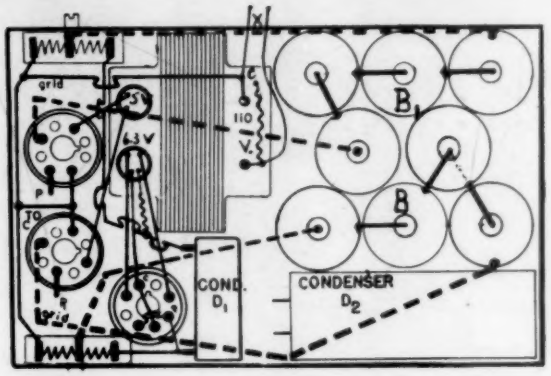
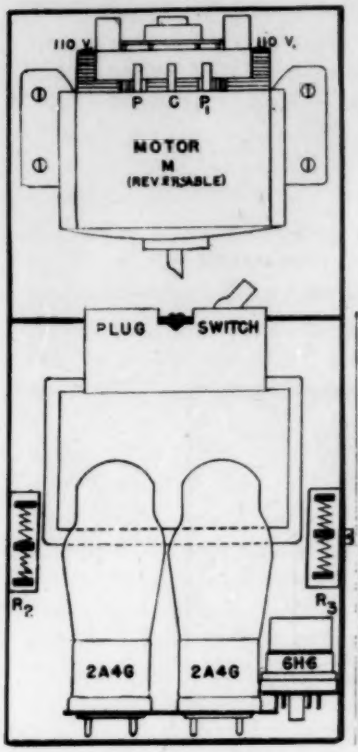


FIG. 2. Pictorial diagram showing spacing and shape of parts and all wiring connections.

through the thyratrons is pulsating DC. It is possible to have the correct value and polarity on R_2 and R_3 and still have the thyratrons fail to respond because they are firing 180° out of phase. To correct this, merely reverse the connections to the 240-volt wires furnishing the plate supply to the 6H6.

Figures 1 and 2 show that the voltage supply for the whole control circuit is taken from X, the output of the variable transformer. This circuit, therefore, controls the voltage to itself which gives increased stability. The exact spacing, relative size, and shape of parts are shown in the figures. Other parts with suitable characteristics can be substituted for those given here, but this may complicate construction somewhat.

The cabinet for this regulator is made from the original Variatron. Simply remove the screws that hold the cabinet together, and raise the top until there is room for the batteries, etc., beneath the variable transformer itself. New screw holes are made at this level, and the screws replaced. The transformer now has, as it were, a basement. The open panels at the side which are formed when the cabinet is extended in this manner should be left open for ventilation. The two similar end panels may be closed if desired.



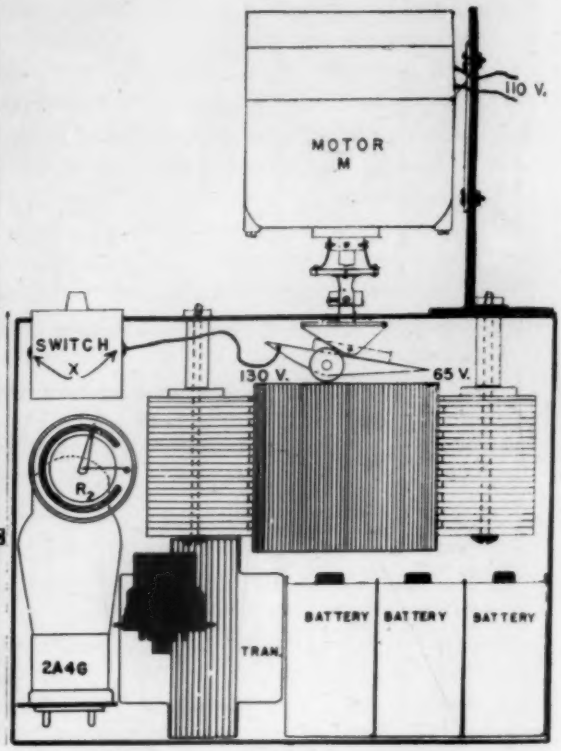


FIG. 3. End view showing mounting of motor, switch, tubes, etc. (left).
FIG. 4. Side view showing shape and spacing of parts (right).

It will be noted that the filaments of the 2A4G tubes are wired in series. This is done to permit use of the one transformer for the whole instrument. If a 2½-volt transformer were used these tubes would, of course, be in parallel. It will also be noted that one leg and the center tap of the high voltage of this transformer is used as plate supply to the 6H6. (See note concerning phase relationships.) Condenser D_1 and resistance R_4 are necessary to the correct functioning of the instrument. Condenser D_2 is used only in case of radio interference.

It has been mentioned above that motor M has two small starting transformers wound within itself, and that the plates of the two thyratron tubes

are in series with them. It might at first seem that the voltage in these transformers would cause current to pass through the tubes and the motor. This is not the case, however, for the transformers have only 20 volts, while the tubes have a voltage drop of 16, leaving only 4 volts, which is insufficient to turn the motor. It will be noted that the cathode of the thyratrons is tied to one side of the 110 line. It is a simple matter, therefore, to tie the center junction (C) of the two transformers to the other side of the 110, and thus have either 90 or 130 volts across the tubes, depending upon whether the transformer voltage is in or out of phase with the line. Either works equally well, but it is necessary with either to protect the tubes and motor from excessive current by means of resistance R_1. This resistance should be 500 to 1,000 ohms, depending on the voltage. Not more than 90 mils should be allowed to pass through the 2A4G tubes.

It is, perhaps, desirable to complete all the wiring except the 6H6 circuit and test out before going further. It should now be possible to proceed as follows: (a) Plug in the 110 source of the variable transformer. The motor should run in one direction, causing the traveling contact of the Variatron either to go off scale at the 130-volt end or move down toward to 65-volt and then stall because of insufficient voltage to run the motor. (It is necessary to connect the shaft of the motor and the Variatron with a friction joint to allow slippage.) (b) Disconnect the plate of the tube which gave the movement and push the traveling contact back to the middle. (c) Reverse the polarity of battery B_1 so that it now is plus instead of minus. The motor should run in the reverse direction showing that the circuit is correct. (d) Reconnect the tube and battery as they were, and connect the 6H6 tube. A check of the voltage from the opposite ends of R_2 and R_3 should show the R_2 end to be minus and the R_3 end plus. A similar check across R_2 should show the end not in common to be minus with the common junction, while the common junction for R_3 should be minus to the other end of R_3. The two batteries (B and B_1) should then be arranged to oppose the polarities of R_2 and R_3.

The action of the complete wiring may be checked as follows: (a) Plug into the 110-volt line again. Turn R_3 so that this tube is held as minus as the battery can make it. R_2 will then be plus from the battery and minus from the 6H6 tube. If the instrument is functioning properly the tube will first fire continuously because of the charge from battery B and then cease to fire because the 6H6 tube has heated up and has made the grid minus. (b) Now adjust R_3. If the traveling contact is at the high voltage end of the Variac, ignore it; but if at the low voltage end, push it to the middle. As resistance R_3 is turned farther and farther, this grid becomes more and more plus, and the tube will first flicker and then fire. The motor will move and the tube cease to fire. Turn R_3 a little farther and its tube will fire again. The motor will move and it will cease to fire. Continue this until the voltage desired is delivered at X. (c) Now adjust R_2 in the same manner until its tube flickers. If both tubes now flicker, the instrument is adjusted and functioning.

If, however, when R_3 is turned as described above, the tube fires and the motor runs, and the tube continues to fire, the circuit is turning the Variatron in the wrong direction. To remedy this, reverse connections P and P_1. To change the voltage output at X it is necessary to adjust the two resistances R_2 and R_3 until the desired output is attained. When perfectly adjusted the tubes will flicker alternately. When once adjusted and thoroughly warmed up, the instrument should maintain well within one volt for many months with no attention whatever. On sudden change of voltage, one tube fires, the motor moves, balance is established, and the tubes flicker. On very slow change of voltage, one tube flickers more rapidly than the other, and balance is established.

Summary

In summary, the characteristics of the instrument are as follows. Amperage range: 5 to 50 amperes depending upon variable transformer used; speed of control: 1 to 5 seconds for sudden 10 volt change; wave form of output: unchanged; range of voltage control: with input voltages of 95 to 120 can be set to give constant output voltages from 105 to 120; load: control is equally accurate for no load to full load for which the transformer is designed; dependability: runs for months with no attention whatever; cost: about $22.00 for 5-ampere model, higher-ampere models increase only with additional cost of variable transformer.

This voltage regulator has been used for many months by the author and has given excellent service. It was of particular value as a voltage source for recording potentiometers. It was likewise used in a series of tests of the ultraviolet output from lamps and it was immediately obvious that the output was highly unreliable without it. Its most interesting general use was to control the entire voltage supply to the laboratory so that all motors, water baths, and incubators were on uniform voltage. Full sized blueprints with detailed instructions and list of parts are available from the author.

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VITAMIN CONTENT OF SOME MATURE AND GERMINATED LEGUME SEEDS[1]

PAUL R. BURKHOLDER AND ILDA MCVEIGH

(WITH ONE FIGURE)

In earlier papers we have reported observations made on some of the changes in B vitamins associated with germination of various kinds of edible seeds (**3, 4**). This work has been continued with seeds of high protein content, including garden pea, Mung bean, and several strains of soybean. Inasmuch as sprouted legume seeds possess protein of high biological value (**6**), and since their palatability is often improved during germination, these materials appear to deserve further serious consideration as a potential source of nutritious food which could easily be made available in large quantities. The present report is concerned with determinations of thiamine, riboflavin, pyridoxine, niacin, pantothenic acid, inositol, vitamin B_c, biotin, and ascorbic acid in mature dry and freshly sprouted seeds of *Pisum sativum* variety Canner King, *Phaseolus aureus* (the Mung bean commonly used in chop suey), and 7 varieties of field type soybean belonging in the species *Soja max*.

Methods

The general methods of investigation have been reported in our earlier papers. Seeds were germinated at 25° C. on moist filter paper in large Petri dishes kept in a darkened room. In most of our experiments seeds were treated with a solution of filtered calcium hypochlorite (5 gm. in 150 ml. H_2O) for 1 hour in order to avoid contamination with microorganisms during germination. One series was sprouted without preliminary treatment with chlorine to determine whether changes in vitamin content would occur similar to those observed in the chlorine-treated seeds. These seeds were washed for 1 hour in running tap water prior to transferring them into Petri dishes.

Various stages of germinated seeds were obtained for analyses on the same day by the simple expedient of soaking the dry materials at 4-, 3-, 2-, and 1-day intervals prior to the date set for the final sampling. Samples of the fresh materials, consisting of 5 individuals of pea and soybean and 20 seeds or seedlings of Mung bean, were taken both for direct assay and for the determination of the constituent dry matter. Weighed amounts of the fresh sprouting seeds were ground in acetate buffer solution adjusted to pH 4.5 for the purpose of obtaining extracts to be assayed for B vitamins.

Dry preparations of papain and Taka-diastase were added to provide each enzyme in amount equivalent to approximately 4 per cent. of the dry weight of the sample. Digestion was allowed to proceed at 37° C. for 24 hours, using a few drops of benzine in the stoppered flasks to inhibit growth

[1] This work was supported in part by the Nutrition Foundation, Inc., New York City.

TABLE I

THE B VITAMIN CONTENT OF DRY AND SPROUTED SOYBEANS AND MUNG BEAN. VALUES ARE EXPRESSED AS MICROGRAMS PER GRAM OF DRY MATTER IN DUPLICATE SETS OF DETERMINATIONS

VARIETIES OF BEANS	THIAMINE		RIBOFLAVIN		PYRIDOXINE		NIACIN		PANTOTHEN		INOSITOL		B_c		BIOTIN	
	DRY	SPROUTED	DRY	SPROUTED	DRY	SPROUTED	DRY	SPROUTED	DRY	SPROUTED	DRY	SPROUTED	DRY	SPROUTED	DRY	SPROUTED
	γ	γ	γ	γ	γ	γ	γ	γ	γ	γ	γ	γ	γ	γ	γ	γ
Peking	15.7	19.6	3.8	5.0	13.3	13.6	21.4	29.9	36.6	34.4	2436	3729	1.89	0.73	0.85	1.10
	16.7	18.0	3.7	4.8	12.0	12.8	22.3	33.2	35.9	34.4	2436	3839	1.83	0.69	0.94	1.08
Manchu	16.0	21.9	3.4	6.0	11.1	17.7	20.3	42.8	19.5	33.1	1855	3944	1.69	1.29	0.76	1.76
	17.7	19.0	3.3	5.3	11.6	16.1	21.7	40.0	19.0	28.6	2086	3441	1.76	1.24	0.80	1.64
Mukden	17.0	14.9	3.4	7.4	11.2	15.2	20.2	46.0	20.3	30.3	2311	3336	1.74	1.03	0.79	1.50
	17.0	17.7	3.4	7.0	10.8	16.6	20.0	48.0	19.6	30.6	2055	3677	1.61	1.37	0.85	1.78
Lincoln	18.9	17.6	3.6	4.9	11.1	15.9	21.3	35.1	22.5	26.8	2549	3340	1.88	1.39	0.80	1.38
	16.5	13.8	3.4	5.1	11.1	14.1	20.0	37.8	21.5	37.8	2516	2852	1.94	1.08	0.70	1.46
Dunfield	13.9	11.9	3.4	4.5	11.5	14.4	20.6	31.2	15.8	18.8	2298	2524	2.01	1.13	0.89	1.49
	13.1	12.5	3.4	4.7	11.3	14.7	20.5	33.7	15.9	22.5	2176	2959	2.07	1.09	0.85	1.54
Illini	18.5	16.8	3.3	4.9	12.6	15.8	21.1	33.4	19.6	26.3	2500	3505	2.16	1.07	0.85	1.74
	18.7	17.9	3.3	5.1	12.1	16.4	20.7	32.2	19.5	26.7	2457	3581	1.99	0.97	0.83	1.72
Anwei	13.4	12.4	4.5	7.0	12.7	17.9	25.9	40.7	18.1	29.9	2276	3383	2.31	0.88	0.70	1.74
	14.0	13.3	4.3	6.3	12.9	16.8	23.2	38.4	17.1	27.3	2112	2877	2.07	1.13	0.63	1.41
Mung	8.8	8.2	3.2	14.1	11.8	13.6	23.4	64.7	16.3	32.5	1812	3635	2.55	1.88	0.25	0.59
	8.2	8.6	2.8	14.7	11.0	15.1	22.8	68.3	16.5	34.8	1887	3663	2.39	2.05	0.20	0.78

of microorganisms. The digested samples were heated in steam at 100° C. for 30 minutes, adjusted to pH 5.5, made to standard volume, and filtered through "supercel" in a Büchner funnel. Riboflavin, niacin, and pantothenic acid were tested with *Lactobacillus casei* in accordance with methods published by R. J. WILLIAMS, *et al.* (**11**). Vitamin B_c was determined with *L. casei* according to the procedure of LANDY and DICKEN (**8**). Pyridoxine was assayed with a yeast growth method employing *Saccharomyces oviformis* (**2**). Biotin was tested with the yeast *S. cerevisiae* 139 using the general method outlined by SNELL, EAKIN, and WILLIAMS (**10**). Inositol was determined with the yeast *Kloeckera brevis* (**5**). Thiamine was assayed by the Phycomyces growth method (**1**). Ascorbic acid was measured by the colorimetric procedure recommended by PEPKOWITZ (**9**). Reduced ascorbic acid was determined directly in the sample ground in metaphosphoric acid, and total ascorbic acid was measured after reduction with H_2S and subsequent displacement of this gas by means of nitrogen (**7**).

Results

The results obtained in duplicate assays for the B vitamins in 7 varieties of soybean and in Mung bean, all treated with chlorine at the time of soaking, are presented in table I. Among the soybeans, values for thiamine appear to be somewhat lower in the varieties Dunfield and Anwei than in the other strains. The thiamine content of Mung beans appears to be considerably less than that found in soybeans. No great change in thiamine occurred during germination of the different kinds of seeds listed in the table.

The riboflavin content was about the same in all varieties of mature soybean with perhaps somewhat higher values for the variety Anwei. During germination, increases in riboflavin were observed in all varieties. Sprouted Mung beans contained almost five times as much riboflavin as the dry mature beans. No great differences in pyridoxine were noted among the various kinds of dry beans; some increases in this vitamin appear in the germinated seeds.

The niacin content is almost uniform throughout the list of dry bean seeds; appreciable gains in niacin were observed in the sprouted materials. The niacin in 4-day-old seedlings compared with nonsprouted seeds approximately doubled in Manchu, Mukden, and Anwei varieties. Sprouted Mung beans almost trebled in niacin content. Pantothenic acid, inositol, and biotin appear to increase in nearly all the sprouted materials, but vitamin B_c is reduced appreciably.

The results of a series of determinations made on germinating seeds of Pisum, Phaseolus, and Soja not treated with chlorine are shown in figure 1. Increases in both reduced and total ascorbic acid were observed in all three seeds, but the increase of total ascorbic acid in sprouted Mung beans was outstanding. Niacin increased during germination in both soybean and Mung bean, but the highest values for this vitamin were observed in sprouted

peas. Peas and Mung beans were notable also for their increases in pantothenic acid during germination. Riboflavin increased in the sprouting seeds of all three, but the highest value occurred in Mung bean sprouts.

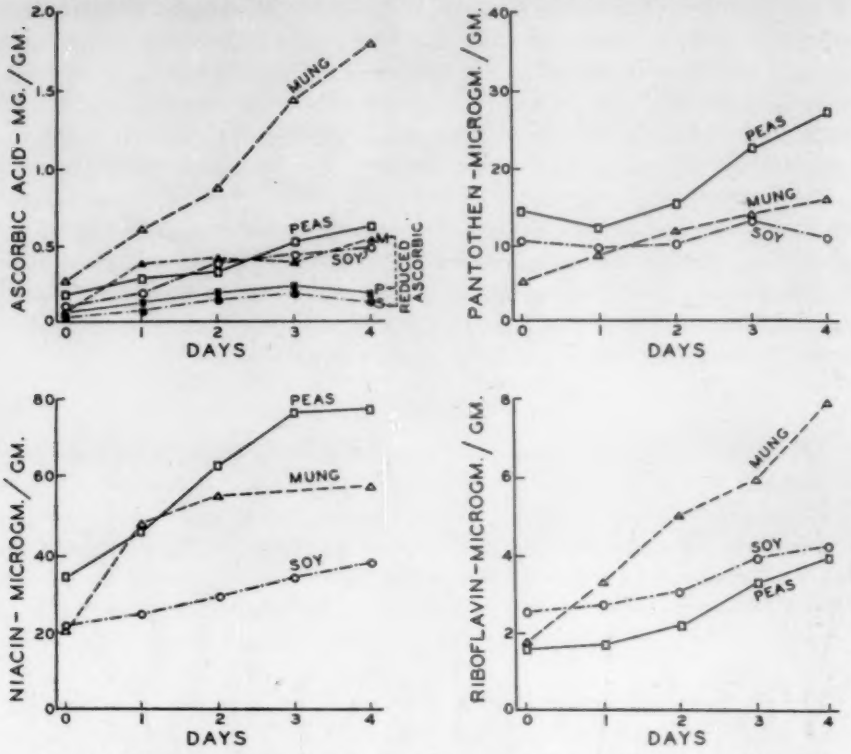

FIG. 1. Vitamin content of mature and sprouted Canner King peas, Mung beans, and Anwei soybeans at 1, 2, 3, and 4 days after soaking in water.

Discussion

Appreciable gains in the concentration of total ascorbic acid, riboflavin, and niacin are associated with germination of peas, soybean, and Mung bean. The calculated changes in dry matter during germination are too small to account for the observed increase of vitamins in these sprouted materials. While some of the B vitamins show increases, values for thiamine generally remain unchanged, and vitamin B_c appears to decline somewhat in these sprouting seeds. The mechanism by which the concentration of some vitamins is augmented in the processes of germination is unknown. It seems reasonable to suppose, however, that increased amounts of vitamins, such as riboflavin and niacin, which are known to be parts of important enzyme systems in intermediate metabolism, might have a profound influence upon the supply of energy in growing tissues of seedlings.

The significance of such data for human and animal nutrition need hardly be stressed. Our investigations indicate that marked increases of

important nutritive substances occur during the sprouting of leguminous and many other kinds of edible seeds. Among the many varieties of soybean which we have used in germination experiments, the garden varieties when sprouted seem to be less satisfactory from the food standpoint than the field types reported in this paper. Among the field varieties the small-seeded Anwei germinated almost as readily as Mung beans. The possible use of sprouted peas, beans, and other seeds in canning and preparation of dehydrated foods would enable these industries to operate on a 12-month basis. If the food value of germinated seeds is to be judged by their content of vitamins and readily available amino acids, then it appears that the common use of sprouts in the diets of Oriental peoples rests on a sound nutritional basis and should be introduced on a wide scale among Occidentals. It is hoped that the data reported here may have significance in academic studies on growth processes of plants and practical value in connection with the use of sprouted seeds for preparing high quality processed foods.

Summary

Microbiological assays were made for thiamine, riboflavin, pyridoxine, niacin, pantothenic acid, inositol, vitamin B_c, biotin, and ascorbic acid in mature and sprouted seeds of *Pisum sativum*, *Phaseolus aureus*, and 7 varieties of *Soja max*. On a dry weight basis, niacin, riboflavin, and ascorbic acid increase greatly during germination. Certain other vitamins show small gains, thiamine generally remains unchanged, and vitamin B_c appears to decrease.

Possible significance of the results for studies on growth processes of plants and in relation to the use of sprouted seeds for preparing high quality processed foods is discussed briefly.

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BRIEF PAPERS

THE USE OF MONOCHLORACETIC ACID TO INCLUDE AMMONIA IN THE CHLORATE METHOD FOR TOTAL NITROGEN IN PLANT TISSUE[1]

E. M. Emmert

The method for determining total nitrogen in plant tissue, described in a previous paper (**1**), is made to include ammonia and is also made more rapid and accurate, by the use of monochloracetic acid (**2**). In the presence of strong alkali, ammonia and monochloracetic react to form glycine which is readily oxidized by the chlorate treatment, the nitrogen passing to nitric acid. Danger of chloric acid remaining in the solution to be tested is also avoided if the reagents are of proper strength. The only pieces of apparatus needed are 30-cc. test tubes, small pipettes and graduates, and a container for boiling water.

Procedure

Place 50 mg. of finely ground (at least 60-mesh; 100-mesh is better), dry tissue or 100 mg. of green tissue, chopped up fairly fine, in the bottom of a 30-cc. test tube. Add 2 cc. of 20 per cent. $NaClO_3$ solution and allow to mix and soak a few seconds. Have boiling water ready. Add 3 cc. of cold 60 per cent. by volume sulphuric acid, washing down any particles of sample. Immediately place the tube in boiling water so that the water is well above (at least 3 inches) the acid level and so that the tube slants considerably. The action will be vigorous and some spattering may occur but it does no harm in the slanting position. Do not shake until the main reaction is over, which takes 3 to 5 minutes. Then shake to wash down particles on the sides of the tube. As soon as the sample is dissolved (5–10 minutes), dilute to 50 cc. with water (25 cc. if N is below 1 per cent., 100 if above 4 per cent.). A few small particles of undecomposed cellulose or lignin in the solution do not make any difference in the nitrogen result, but of couse the main bulk of the sample must be oxidized. The solution should be yellow from excess chlorate. If it is not, insufficient chlorate was used.

Place 1 gm. of monochloracetic acid in a 30-cc. test tube, add 0.5 cc. of the yellow oxidized solution and shake occasionally until most of the monochloracetic acid is dissolved. Add 1.2 cc. of 40 per cent. NaOH and shake until all the monochloracetic acid dissolves. Add 0.5 cc. of 40 per cent. $NaClO_3$ solution. Hold the tube in a test-tube holder and add 2.5 cc. of fuming sulphuric acid (15 per cent. SO_3) slowly enough to avoid excessive boiling. Too rapid addition may cause loss. The solution should remain a strong yellow color. Shake and blow out the fumes with a bent glass tube until the color fades to a faint yellow. This takes just a few seconds. Cool

[1] The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the director.

to about room temperature, add 3 cc. of phenoldisulfonic acid, and shake well. If an orange color forms, the fuming acid is weak. The solution should remain almost colorless. Add 15 to 20 cc. of water and transfer to a larger container for neutralization with 40 per cent. NaOH. Add a small excess of NaOH; the end point is recognized by the formation of the yellow nitrate color. Dilute to such a volume that the color is near that of a standard solution containing 0.0005 mgm. of N per cc. This standard is made in the usual way for nitrate determinations, with the exception that it is weaker than usual and treated like the unknown. The reason for dilution is that the photo-electric colorimeter readings are more accurate on weak solutions. A blank must be carried through along with the unknown and the reading from it subtracted from those of the unknown and standard.

Results

Samples of tobacco stalks and of straw, on which N had been determined by the Kjeldahl method, were tested. Small amounts of NH_3 were added and the increased percentage calculated. The results are given in table I.

TABLE I

PERCENTAGE OF NITROGEN FOUND IN THE AIR-DRIED TISSUE; WITHOUT AND WITH ADDED AMMONIA SALT

	Sample number			
	6	8	10	11
	Material			
	Wheat straw	Tobacco stalks	Tobacco stalks (Leached through May)	Tobacco stalks (Leached through June)
	%	%	%	%
By Kjeldahl method	0.90	2.73	1.71	1.59
By Chlorate method	0.910	2.760	1.720	1.600
	0.905	2.730	1.750	1.620
Mean	0.908	2.745	1.735	1.610
Same plus Ammonium Salt	0.985	2.820	1.797	1.700
	0.984	2.833	.....	1.680
Mean	0.985	2.826	1.797	1.690
Ammonia N recovered	0.077	0.081	0.062	0.080
Ammonia N added	0.082	0.082	0.082	0.082
Percentage of added N recovered	94	99	76	98

The results are a little higher than by the Kjeldahl method, but are close. The recovery of ammonia was good. Larger additions of NH_3 were tried on 2 aliquots from sample no. 8 which contained the highest percentage of nitrogen. If recovery of large additions of ammonia to this sample, already high in nitrogen, could be secured, it would be proof that ammonia was oxidized even in presence of other nitrogen. One-tenth cc. of a standard ammonia solution containing 0.1 mgm. NH_3 per cc. was added to the aliquots

giving an addition of 0.01 mgm. to each 0.5-cc. aliquot from sample no. 8 or an equivalent of 1 mgm. of NH_3 to the 50-mgm. sample. Aliquots without ammonia addition were run at the same time and the nitrogen in them subtracted from the ones to which NH_3 was added. In one case 0.00824 mgm. of nitrogen was recovered and in the other, 0.00828. When this is converted to ammonia, we get 0.1001 mgm. of NH_3 in one and 0.01005 mgm. in the other. This gives further proof that NH_3 is completely oxidized in the procedure. Since no. 10 showed incomplete recovery in the table, an addition like that on no. 8 was made to it and the recovery was 0.01012 mgm. of NH_3 showing that the incomplete recovery on no. 10 must have been due to some experimental error.

These results warrant further trial of the method.

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ALUMINUM TOXICITY: MICROCHEMICAL TESTS FOR INORGANICALLY AND ORGANICALLY BOUND PHOSPHORUS[1]

KENNETH E. WRIGHT

Previous papers (**4**, **5**) by the writer have indicated that aluminum toxicity in plants may be caused by the internal precipitation of phosphorus by aluminum, thus making phosphorus unavailable for plant metabolism. By microchemical tests on fresh sections of roots further evidence supporting the suggestion that phosphorus is precipitated internally by aluminum is recorded in this paper.

Procedure

Barley seeds were germinated on a disc in distilled water, and at the end of ten days the plants were transferred to culture solutions. Each culture solution contained 12 p.p.m. of phosphorus from $CaH_4(PO_4)_2 \cdot H_2O$. To one of them 8 p.p.m. of aluminum were added from $Al_2(SO_4)_3 \cdot 18\,H_2O$. Five weeks later sections of roots were cut on a sliding microtome. Tests were made for inorganically bound phosphorus according to HANSEN's method as described by SCHNEIDER-ZIMMERMAN (**3**), and for organically bound phosphorus according to KLEIN (**1**). An outline of the methods follows.

INORGANICALLY BOUND PHOSPHORUS

A drop of ammonium molybdate solution, made by dissolving one gram of ammonium molybdate in 12 ml. of nitric acid of specific gravity 1.18, is placed on the sections. The formation of small yellow drops with a black border which gradually change to yellow crystals, mostly cubes and octahedrons, indicates the presence of inorganically bound phosphorus.

ORGANICALLY BOUND PHOSPHORUS

1. Fix the sections for two hours in 95 per cent. alcohol.
2. Wash for forty-eight hours in distilled water to remove soluble phosphates. If a test with the molybdate reagent is positive, do step 3; if negative, omit step 3.
3. Wash the sections with 30 per cent. nitric acid and then with distilled water until a test with the molybdate reagent is negative.
4. Place the sections for two hours in a catalyzer of ferrous nitrate solution to which one drop of fuming nitric acid per 100 ml. of solution has been added. The ferrous nitrate reagent was made by diluting 25 ml. of a ferrous nitrate solution of specific gravity 1.20 to 100 ml. with distilled water.
5. Place the sections on a slide and add one drop of 30 per cent. H_2O_2 for one minute. Then warm gently over a flame just to dryness. This latter step is a modification of the original method which directed transfer of the

[1] Contribution no. 664 of the Rhode Island Agricultural Experiment Station, Kingston, R. I.

sections from the 30 per cent. H_2O_2 after one minute to dilute H_2O_2 for one to two hours, after which the H_2O_2 was absorbed by zinc dust. When transferred from the 30 per cent. H_2O_2 variable results were obtained because of the diffusion of freed phosphorus from the section into the surrounding 30 per cent. H_2O_2.

6. Treat the sections with the molybdate reagent. What was formerly organically bound phosphorus is now free and gives a positive test.

7. By means of crystal counts the relative amounts of inorganically and organically bound phosphorus may be estimated.

Results

The molybdate reagent placed directly on sections of the roots from the aluminum-free culture solution gave a negative test even after standing overnight. In the size of sections used this result shows that the amount of inorganically bound phosphorus present was below the threshold of the test. The reagent placed directly on similar-sized sections of roots from the aluminum culture solution gave an immediate positive reaction. Crystals were numerous and variable in size, small ones predominating. They were scattered throughout the reagent, but were more concentrated at the periphery of the field. In these mounts a crystal count for quantitative comparisons was not feasible. To the unaided eye there was a dense yellow precipitate visible on the slide.

After washing the root sections from the aluminum culture solution in 30 per cent. nitric acid there was a negative test with the molybdate reagent showing the disappearance of the inorganically bound phosphorus. Likewise there was a negative test for aluminum with alizarin (**2**), whereas sections from these same roots placed directly in alizarin gave a positive test for aluminum. From the simultaneous disappearance of large amounts of inorganically bound phosphorus and of aluminum, coupled with the apparent lack of inorganically bound phosphorus in the control roots, it is inferred that the inorganic phosphorus compound in the roots to which aluminum was available might well be aluminum phosphate.

After treatment with 30 per cent. H_2O_2 the roots from the aluminum-free culture solution gave a positive test with the molybdate reagent. The reaction was slow, and the crystals were large. A faint yellow could be discerned by the naked eye. The test with the roots from the aluminum culture solution was also positive. The reaction was rather rapid, and crystals were small. Again a faint yellow could be detected with the unaided eye. Because of the heterogeneous distribution of different-sized crystals in the mounts used, no satisfactory crystal count could be made for relative quantitative estimates of organically bound phosphorus.

Summary

Microchemical tests for the estimation of inorganically and organically bound phosphorus in fresh sections of plant material are described. The

application of these tests to barley plants grown in culture solutions with and without aluminum shows abundant inorganically bound phosphorus in the roots grown in contact with aluminum, and little or none in the roots from the culture solutions lacking aluminum. This result indicates a possible combination of phosphate with aluminum in those plants where aluminum was supplied in the culture solution.

The writer wishes to express his thanks to Dr. Homer C. Sampson of Ohio State University for his suggestions and cooperation in the use of these tests.

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PRE-STORAGE CARBON DIOXIDE TREATMENTS FOR CONTROL OF APPLE SCALD[1]

S. A. Pieniazek

Apple scald is a non-parasitic storage disease that causes great losses to growers and distributors of susceptible varieties. It is believed to be caused by volatile products given off by the fruit (**1**). Partial remedies for the disease have been known for some time. Delayed picking was suggested by Powell and Fulton (**3**) oiled paper wrappers by Brooks, Cooley, and Fisher (**1**), and waxing and air conditioning by Smock and Southwick (**4**).

Some work has also been done on control of scald by storing fruits in pure oxygen (**3**), pure nitrogen (**3**), pure carbon dioxide (**1**), and in tightly sealed containers (**1, 2**) for various periods of time. The trials were made both at the beginning and toward the end of the storage season. The work was done on the assumption that a change in fruit metabolism can be thus induced resulting in elimination of the scald causing factors. Some of the treatments prevented scald but were not considered practical.

Experiments at Rhode Island Agricultural Experiment Station show the possibility of scald control by short exposures of apples to atmospheres of 30 and 60 per cent. of carbon dioxide at the beginning of the storage season.

Rhode Island Greening apples were used for the experiment. They were picked on September 1 and 21, 1944. Some of the fruits were treated immediately after picking, others after a month in storage. The treatments made at 32° F. in a storage room partially filled with apples gave most promising results. They consisted of passing a slow stream of air containing 30 and 60 per cent. of carbon dioxide through five gallon containers filled with fruit for 3, 6, and 10 days, respectively.

The fruits, after the treatments were over, were stored together at 32° F. On January 28, 1945, they were taken out of storage, some of them subjected to a pressure test, and the remainder placed in a room at 65° to 70° F. They were examined for scald a week later.

Check fruits picked on September 1 had 78 per cent. scald. Fruit picked at the same time and treated immediately with 30 and 60 per cent. carbon dioxide, respectively, gave 100 per cent. control for the 3-, 6-, and 10-day treatments. Apples treated after a month of storage had from 6 to 24 per cent. scald.

Check fruit picked on September 21 had 50 per cent. scald. Immediate and delayed treatments gave satisfactory results with scald ranging from none to 20 per cent. The flavor of fruit treated at storage temperature was in no case affected adversely. The treated apples, however, were harder and greener than the check.

[1] Contribution no. 668 of the Rhode Island Agricultural Experiment Station, Kingston, Rhode Island.

The same carbon dioxide treatments were given to fruits at room temperatures for 1, 2, 3, and 4 days. The results were erratic and are not considered conclusive. Flavor was slightly affected by the four-day treatment.

In addition to carbon dioxide treatments the apples were tightly sealed in five gallon jars prior to storage for 3, 6, and 10 days at 32° F. and for 1, 2, 3, and 4 days at room temperature. These treatments gave partial to complete control of scald, depending on length of treatment. No off-flavors were noticed except possibly in the four-day treatment at room temperature.

Experiments are being planned for the next storage season in which dry ice will be used for short periods of time as a source of carbon dioxide in storage rooms for control of scald.

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NOTES

Editorial Board of Plant Physiology.—Professor Paul J. Kramer was appointed to the Editorial Board of Plant Physiology by the Executive Committee of the Society to fill the vacancy created by the recent death of Dean C. B. Lipman.

Plant Physiology Monographs.—The American Society of Plant Physiologists recently voted to establish an Editorial Board to handle the publication of monographs by adoption of the following constitutional provision: "The Editorial Board of the American Society of Plant Physiologists' Monographs shall consist of an Editor-in-Chief and four members appointed by the Executive Committee, one each year for a term of five years, and of the Editor-in-Chief of Plant Physiology. It shall be the duty of the board to select the titles of monographs to be published, to determine the fiscal and editorial policies to be followed, and to advise in the selection of key contributors."

In harmony with the above provision, the Executive Committee has created a revolving fund for publication of future monographs and has selected the following Monograph Editorial Board to supervise the monograph series of the Society: W. E. Loomis, Editor-in-Chief, 1950; D. R. Hoagland, 1945; F. W. Went, 1947; J. W. Shive, 1948; Gordon MacKinney, 1946; and W. F. Loehwing, ex-officio, 1949.

Daniel T. Macdougal.—The American Society of Plant Physiologists conveys its felicitations to Dr. D. T. Macdougal on the occasion of his 80th anniversary on March 16, 1945. Since retirement Doctor Macdougal has continued his research work at Carmel, California. It is with distinct pleasure that the Society takes this opportunity to convey its good wishes to one of its distinguished Charles Reid Barnes life members upon attaining the milestone of four score years.

N. A. Maximov.—Dr. Maximov, a corresponding member of the American Society of Plant Physiologists and the American Botanical Society, would like to receive again reprints of papers in plant physiology from colleagues in the United States and Canada. Shipment is now possible because of the establishment of more normal postal service to the U.S.S.R. His address is: Academy of Sciences, Bolshaia Kalushkaia 13, Kv. 16, Moskva 49, U.S.S.R. Professor Maximov is now preparing the 8th revised Russian edition of his popular *Textbook of Plant Physiology*. Two English editions of it have been issued in the United States. It has been translated also into the Ukrainian, White Russian, Georgian, and Usbekian languages. A Spanish edition of this text is being printed in Argentina. Dr. Maximov is also working on the second edition of his book, *The Plant in Relation to Water*, the first edition of which was translated into English in 1929.

Burton Edward Livingston.—The American Society of Plant Physiologists felicitates DR. BURTON E. LIVINGSTON on the occasion of his Seventieth Anniversary on February 9, 1945. The society takes this opportunity to express its appreciation for his continued interest in its success and gratefully acknowledges the numerous services which he has rendered ever since its organization. The society has been fortunate in having the professional judgment of a distinguished scholar and a capable executive at its disposal for nearly a quarter of a century.

In the early years of the society's existence, DR. LIVINSTON gave the same meticulous care to its affairs as he had to his own botanical research with the result that highly successful fiscal and administrative policies were promptly established for the new organization. The soundness of the constitution and the investment program as originally drafted by DR. LIVINGSTON is demonstrated by the fact that they both still remain substantially unaltered. His election to the presidency of the society in 1934, to the first BARNES honorary life membership, to the executive committee, and to the editorial board of PLANT PHYSIOLOGY were not merely recognition of his eminence as a scholar but an appreciative tribute to his unselfish service to science. DR. LIVINGSTON'S career has been a life-long record of scholarly productivity coupled with unusual foresight in promotion of professional ideals which have materially enhanced the prestige and effectiveness of the plant sciences. Release from teaching responsibilities upon retirement from his professorship has permitted him to turn his undiminished energy even more effectively to the improvement of professional organizations of importance to all scientists. His many years of executive work in the American Association for the Advancement of Science and numerous other professional organizations constitute a remarkable record of public-spirited service. Those who have been privileged to work with DR. LIVINGSTON have been impressed by his sterling character, his innate generosity, and the breadth of his understanding both of individuals and problems. The American Society of Plant Physiologists conveys its greetings and appreciation to DR. LIVINGSTON upon his seventieth birthday.

Rufus Percival Hibbard.—The American Society of Plant Physiologists conveys its congratulations to DR. R. P. HIBBARD on his seventieth birthday on April 2, 1945. The society takes this occasion to recognize the enduring support of one of its founders. DR. HIBBARD actively participated in organization of the society and in the first years of its existence helped carry the financial burdens incident to the establishment of PLANT PHYSIOLOGY. As the first vice-president and second president of the society in 1926, he contributed greatly to the success of the society and its official journal. In addition to his two years of service as an executive of the society, DR. HIBBARD for many years contributed generously in time and substance as a member of the executive committee and editorial board.

DR. HIBBARD'S rich experience, broad scientific interests, and persuasive personality were helpful not only in the formative phases of the society's

history but also aided its subsequent growth. His services to the society reflect the high principles, proficiency, and unselfish devotion which characterize his professional career. In his community, as in his scientific work, DR. HIBBARD has been a public-minded citizen who enjoys the esteem of his neighbors and professional associates. After his retirement in 1940, he voluntarily assumed and still carries responsibility for a portion of the training program for the Army Air Forces at Michigan State College in connection with which he teaches physics. His own published work and that of his numerous graduate students, to whom he has given generously of his time and effort, reflect DR. HIBBARD'S stimulating spirit. His many friends and associates join in a message of felicitation to him upon the occasion of his seventieth anniversary.

William Edward Tottingham.—The American Society of Plant Physiologists records with sorrow the death on March 2, 1944, of one of its founders. DR. TOTTINGHAM assisted in the organization of the society and its journal, PLANT PHYSIOLOGY. For many years he also served on the Executive Committee and the Editorial Board. He was elected Vice President of the Society in 1927 and President in 1931.

PROFESSOR TOTTINGHAM, son of EDWIN H. and MARIA SHEARER TOTTINGHAM, was born at Barnardston, Massachusetts, November 2, 1881. PROFESSOR TOTTINGHAM received his early education in his home community and was graduated from Massachusetts State College, Amherst, Massachusetts, in 1903. He served for one year on the staff of the New York State Agricultural Experiment Station at Geneva and in 1906 joined the University of Wisconsin staff as instructor in agricultural chemistry. He received his Master's degree after coming to Wisconsin and later returned to Johns Hopkins University for his Doctorate. As a member of the University of Wisconsin faculty PROFESSOR TOTTINGHAM worked quietly but effectively in his chosen field of plant biochemistry. He was particularly interested in those problems which would be of practical value to the farmers of the state and of the nation.

In 1914, he published an extensive paper on "A Quantitative Chemical and Physiological Study of the Nutrient Solutions for Plant Culture" which has become a standard reference in plant chemistry. Much of his time was devoted to studies on winter hardiness in plants and the effect of light on nitrate absorption. More recently he studied with increasing interest the complex and difficult problem of blackening of potatoes during cooking, a problem which not only has definite economic significance to the grower and consumer alike, but involves intricate processes of biochemistry. In 1934 he published his *Plant Biochemistry*, at that time the only book of its kind in the English language in which the subject of plant biochemistry was treated comprehensively. Recently arrangements were being made to have the book translated into Portuguese. A few hours before his death he was completing a technical paper on some of his recent investigations. Students

trained in his laboratory, many now distinguished in the field of biochemistry, remember him as a patient and sympathetic advisor, a staunch friend, and able teacher.

In addition to his University duties, PROFESSOR TOTTINGHAM took an active interest in church and community affairs. He gave generously of his time and resources whenever he could. Music was part of his daily life and he shared this enjoyment with his family, friends, and members of his musical organizations. He will be keenly missed by a host of friends who had learned to love and admire his kindness and sympathetic understanding. His courage, sincerity, and steadfastness of purpose were admired by all who knew him. At the time of his death, DR. TOTTINGHAM was in his 38th year of service at the University of Wisconsin. He is survived by his widow, ETHEL LERCH TOTTINGHAM, and two children, ELAINE and ROBERT.

Howard Henry Zimmerley.—Director of the Virginia Truck Experiment Station since 1932, DR. ZIMMERLEY died of a heart attack on October 15, 1944. He enjoyed an unusually wide acquaintance with farmers, agricultural workers, and industrialists; with the universal esteem accorded him, his place will be difficult to fill. His death at 54 years of age is a distinct loss to agricultural science and biology.

DR. ZIMMERLEY was born in Erie, Pa., February 26, 1890. He attended Pennsylvania State College, graduating in 1912. He received his doctor's degree at the University of Maryland in 1931, and an honorary Doctor-of-Science degree from Clemson College in 1937. Before he became director in 1932, he had served for 15 years as assistant horticulturist and horticulturist at the Virginia Truck Experiment Station. He took a prominent part in fertilizer research and was general chairman of the National Joint Committee on Fertilizer Application in 1937 and 1938. He also served as chairman of the committee representing the American Society for Horticultural Science on the Joint Committee on fertilizers since 1933. He was president of that Society in 1935 and served as treasurer of the National Joint Committee on Nitrogen Utilization. Soon after its formation, DR. ZIMMERLEY joined the American Society of Plant Physiologists which mourns the loss of a distinguished member of long standing.

J. H. Priestley.—The announcement in NATURE of the recent death of PROFESSOR J. H. PRIESTLEY, Head of the Botany Department of the University of Leeds, comes as a shock to his many friends in this country. His passing removes from the plant sciences a gifted and inspiring teacher, an original investigator, and a stimulating writer. During the period 1926–28 it was the writer's great privilege to be associated with him as a post-graduate student and the following comments are a brief tribute to the personality and philosophy of a unique figure in modern botany.

In his contact with undergraduates, especially in the lecture room, PRIESTLEY brought a fresh new viewpoint to the presentation of botani-

cal problems. No formalist, PRIESTLEY refused in any sense to separate anatomy from physiology. In his own words, a structure is to be explained "as the inevitable outcome of the reaction between earlier developmental stages and its environment." In this sense, "causal anatomy" became a hopeful line of inquiry and was presented with a buoyancy and an enthusiasm which affected all his listeners. As illustrative of his conviction of the morphogenetic importance of the terminal meristems in plant, PRIESTLEY almost invariably began his lecture by drawing on the blackboard an outline of the shoot apex, regardless of whether his main concern at that period was the endodermis, cork, or the vascular system! To him the apex represented the beginning of the series of causally related processes which culminate in the matured structure of the plant. PRIESTLEY'S relations with graduate and advanced students were stimulating to an unusual degree. At the end of a "conference" one fully realized the keenness of his mind which always sought the "why" as well as the "how" in any problem. Although fully aware of the provisional character of many of his ideas, PRIESTLEY maintained a vigorous intellectual optimism towards all the botanical inquiries which engaged his alert mind. Shortly before his untimely death, the writer received a cordial letter from him discussing with his usual enthusiasm the difficult problem of the growth of laticiferous ducts. Written when he was far from well, this final message emphasizes clearly his indomitable spirit. His death is a source of deepest regret to all who knew him as a man and as a scientist.—ADRIANCE S. FOSTER.

Hunger Signs in Crops.—A second printing of this publication, edited by GOVE HAMBIDGE and published by the American Society of Agronomy and the National Fertilizer Association, is now in press. The first printing of 14,000 copies published in 1941 is now practically exhausted and a second printing of 8,000 copies is being published. The new impression may be ordered directly from the printer, Judd and Detweiler, Inc., Florida Avenue and Edkington Place, N.E., Washington 2, D. C., or through local bookstores. The price per copy of the new issue is $2.10. The small remaining number of copies of the first edition continues to sell at $2.50.

Manometric Techniques and Related Methods for the Study of Tissue Metabolism.—This laboratory guide is the work of W. W. UMBREIT, R. H. BURRIS, J. F. STAUFFER, and nine other contributors on the University of Wisconsin staff; it contains 198 pages with working descriptions of methods. The book is a laboratory companion to the earlier volume on *Respiratory Enzymes*, incorporating successful procedures developed by the authors in their own laboratories. Methods described involve use of a minimum of laboratory research equipment. Directions embody details, precautions, and hints designed to insure success and precision in delicate quantitative procedures.

The manual describes the Warburg Constant Volume Respirometer, the direct and indirect methods of CO_2, manometric techniques, use of the differential manometer in photosynthesis studies, methods of tissue preparation including homogenates, estimation of dehydrogenase by the Thunberg technique, and manometric determination of enzyme systems and metabolites. Procedures are given for preparation and analysis of phosphorylated and other important intermediates and metabolites. Each chapter contains a list of pertinent citations. The book lists at $3.50 and the publisher is the Burgess Publishing Company, 426 So. Sixth Street, Minneapolis, Minnesota.

Problems in Tree Nutrition—This 184-page volume by M. C. RAYNER and W. NEILSON-JONES of Bedford College, University of London, was published in 1944; it is concerned with the mycorrhizal habit in relation to forestry and soil fertility. The book contains the results of the comprehensive and fruitful studies of the authors on tree mycorrhiza and their relation to the fertility of forest soils. The various chapters treat of research on pines and other conifers, their mycorrhiza, organic composts in relation to growth of young trees, and biological aspects of soil fertility. A bibliography of 58 references, an eight-page glossary of terms, and a general index are included. The publishers, Faber and Faber Ltd., 24 Russell Square, London, W.C.I., list the price of the book at 12s. 6d, net.

The Permeability of Living Cells.—This is a lithoprint by SUMMER C. BROOKS and MATILDA MOLDENHAUER BROOKS of the University of California. It is a production in English of Volume 19, 1941, of the *Protoplasma Monographs*. This 396-page volume is an experimental approach to cell permeability with treatment of rates of diffusion, effects of radio-active elements, and respiration and ion effects. An introductory chapter discusses membranes and general aspects of permeability. Water relations, osmotic effects in plant and animal cells, cell permeability to gases, dyes, strong and weak electrolytes are described in 13 chapters. The volume contains a table of symbols, more than 1200 bibliographical citations, and separate author and subject indexes. The monograph is a comprehensive and authoritative compilation of major contributions in the field of cell permeability and will be welcomed by all physiologists. The volume as originally published by Borntraeger of Berlin in 1941 sold for $9.60. The cost of the lithoprinted publication is $5.00, sold by J. W. Edwards Publishing Company of Ann Arbor, Michigan.

A Manual of Soil Fungi.—This 392-page volume by JOSEPH C. GILMAN, Department of Botany, Iowa State College, is designed to enable investigators to identify soil fungi. The volume assembles descriptions of fungi from numerous sources and provides comprehensive keys with many detailed drawings for identification. Considerable attention has been given to nomenclature and a conservative taxonomic treatment has been followed

especially for those genera and species whose classification is now in process of revision. The arrangement of orders, families, and genera of fungi is in general that of LINDAU in ENGLER and PRANTL, *Die natürlichen Pflanzenfamilien,* while more recent treatments are followed for Mucorales, Saprolegniaceae, Penicillium, Aspergillus, and Fusarium. Actinomyces and Zoopagaceae have been omitted. The book comprises the following chapters: Introduction, Key to the Families, Phycomycetes, Ascomycetes, Fungi Imperfecti, and Mycelia Sterilia. It contains a bibliography of 169 references, a six-page glossary of terms, and a very complete index. The volume lists at $5.00 and is available through the Collegiate Press, Ames, Iowa.

The Chemistry and Technology of Food and Food Products.—Volume 2, 1944, of this work contains contributions of 27 collaborators under editorship of M. B. JACOBS of the Bureau of Food and Drugs, New York City Department of Health. The second volume comprises 890 pages of four major units on operations and processes, sanitary and quality control, preservation, and production. Each unit consists of a series of separate chapters written by experts. Unit operations, food supervision by governmental agencies, grading machines, insecticides, rodent control, fungicides, dehydration, industrial waters, preservation, containers and packaging, cereal, bakery, sugar, cacao, milk, meat, beverage, fat, and fruit products are among the topics described in the 15 chapters of the second volume.

The new book follows the excellent format of the first volume which permits rapid reference use and easy reading. Numerous tables, statistical data, flow diagrams, and photographs of equipment and materials contribute to clarity and value of the information. A very comprehensive cross-reference index of 55 pages is included. The two volumes contain information of value to biochemists and plant physiologists, particularly in relation to methods and formulae. The set of two volumes is priced at $19.00, with individual volumes at $10.50 each purchasable from the Interscience Publishers, Inc., 215 Fourth Avenue, New York City 3, N. Y.